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STUDIES

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RECORDS OF DECAPOD CRUSTACEA FROM BRITISH COLUMBIA[1]

T. H. BUTLER

Abstract

Axiopsis spinulicauda was taken in Holberg Inlet and Barkley Sound at 50 to 70 fathoms, and is a new record for British Columbia. The typically oceanic *Eualus macrophthalmus* was taken in Howe Sound at 60 to 75 fathoms and *Lopholithodes foraminatus* at localities north to Hecate Strait. *Cancer gracilis* was found in deeper water (74 to 78 fathoms) than heretofore and *C. magister*, at 58 to 98 fathoms.

Exploratory fishing conducted by the Fisheries Research Board's Biological Station at Nanaimo in 1954, 1955, and 1959 has contributed to the knowledge of the decapod crustacean fauna of the west coast of North America. Shrimp trawl tows were made by the station's vessel *Investigator* No. 1, and the chartered trawlers, *Yuri M* and *Glendale V*. Two species were collected by the commercial shrimpers, *Seven Seas II* and *Dodie M*, in 1949 and 1959. In all, the ranges of five species have been extended. The distribution lists of Rathbun (5), Schmitt (7), and Hart (1) have been consulted.

Specimens of *Eualus macrophthalmus* and *Axiopsis spinulicauda* are in the National Museum, Ottawa.

Family HIPPOLYTIDAE

Eualus macrophthalmus (Rathbun)

Range.—Unalaska to Point Sur, California, 60 to 636 fathoms.

Present collection.—Near Britannia Beach, Howe Sound, 60 to 75 fathoms on June 1, 1949; nine ♀, 53–65 mm in length from tip of rostum to end of telson.

Remarks.—The species was described by Rathbun (4) as *Spirontocaris macrophthalma*, and later Holthuis (2) in his revision placed it in *Eualus*. Previously *E. macrophthalmus* had been taken only from the archibenthic zone, at 178 fathoms and deeper. This occurrence in a coastal inlet at 60 fathoms extends its range considerably. The present specimens represent a second British Columbia record; the first was by the *Albatross* in 1888, about 60 miles northwest of Cape Scott, Vancouver Island, in 204 fathoms.

Family LITHODIDAE

Lopholithodes foraminatus (Stimpson)

Range.—Hecate Strait, British Columbia, to San Diego, California.

Present collection.—From Esteban Point to Cox Island, west coast of Vancouver Island, 69 to 83 fathoms, at four localities, June 30 to July 3, 1955. Smith Sound, 59 to 63 fathoms on August 3, 1955. North of Banks Island, Hecate Strait, 48 to 60 fathoms, July 25, 1959; carapace widths of five ♂, 62–159 mm and three ♀, 101–132 mm.

[1]Manuscript received September 30, 1960.
Contribution from the Fisheries Research Board of Canada Biological Station, Nanaimo, B. C.

Remarks.—Hart (1) reported the northern limit of this species at Clayoquot Sound and Nanoose Bay, Vancouver Island. The known range now is extended northward by approximately 300 miles.

Family AXIIDAE

Axiopsis spinulicauda (Rathbun)

Range.—Holberg Inlet, British Columbia to Bodega Head, California, 39 to 140 fathoms.

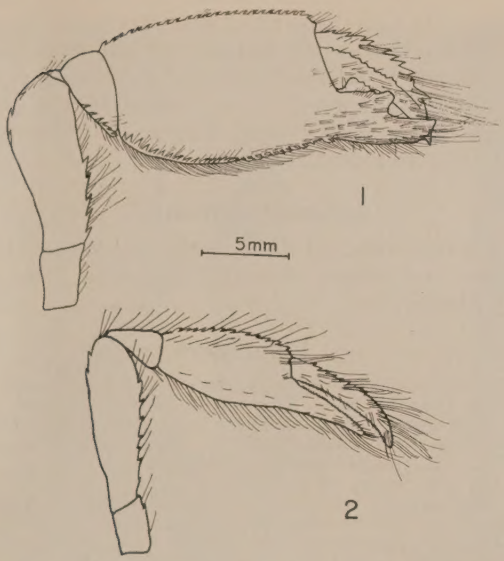
Present collection.—Holberg Inlet, 59 to 61 fathoms, July 18, 1954; one female. Barkley Sound, 50 to 70 fathoms, February 10, 1960; one female. Through the kind permission of Dr. Belle A. Stevens, two records from Puget Sound are included: Rolling Bay 100 to 140 fathoms, July 10, 1940, an ovigerous female; Great Bend off Potlatch, Hood Canal, 39 to 42 fathoms, July 2, 1932.

Remarks.—This species is recorded from British Columbia for the first time. The holotype, a female, was taken by the *Albatross* off Bodega Head, California, at 62 fathoms, March 28, 1890, and described by Rathbun (4) as *Axius spinulicauda*. Later Schmitt (7) considered that it belonged to the genus *Axiopsis*. The present specimens correspond closely to Rathbun's description. Slight differences are as follows: branchial groove on carapace almost as deep as cervical groove; the right and left margins of the rostrum have five

TABLE I

Axiopsis spinulicauda. Lengths and armature of segments of chelipeds

Cheliped	Segment	Lengths (mm)		Armature
		Holberg Inlet specimen	Barkley Sound specimen	
Right (large)	Ischium	3.4	6.3	One to two conspicuous spines on distal half of lower margin
	Merus	9.3	13.6	Four to five conspicuous spines, evenly spaced on lower margin
	Carpus	3.7	5.5	Seven to 10 spines along lower margin
	Manus	12.1	19.0	Upper margin with 24–29 spines to insertion of dactylus; lower margin with 26–28 spines, some curved towards outer face of manus
	Propodus	7.5	11.5	Upper edge curved with two subequal teeth in proximal half and small spine near tip
	Dactylus	9.7	15.1	Eleven to 12 spines along upper margin; longitudinal ridge along outer margin; lower edge curved and smooth, except large blunt tooth near insertion
Left (small)	Ischium	3.5	6.9	One to two conspicuous spines on lower edge
	Merus	8.9	13.4	Two spines, distal end of upper margin; four or seven spines evenly spaced on lower margin
	Carpus	3.5	6.0	Lower edge with 11 blunt spines (larger specimen only)
	Manus	6.0	13.6	Upper edge with 12–20 spines, increasing in size towards insertion of dactylus; lower edge with 21–22 spines
	Propodus	4.5	12.0	Upper edge with 24–38 spines; 2 spines on lower edge continuation of series on manus
	Dactylus	7.0	14.6	Upper edge with 9–11 spines; lower edge with 38–47 spines



FIGS. 1 and 2. *Axiopsis spinulicauda.* Fig. 1. Outer face of right (large) cheliped. Fig. 2. Inner face of left (small) cheliped.

and four or seven spines, respectively; spines are lacking on the outer carinae of the exopods of the uropods of the smaller female. Figure 3 is a photograph of the specimen from Barkley Sound which had most of the pereiopods broken off when caught. The holotype lacked the first pair of pereiopods. Fortunately, the present specimens have these, which are chelate and unequal. Lengths and armature of segments of the chelipeds are given in Table I. Line drawings of the chelipeds of the Holberg Inlet specimen are shown (Figs. 1 and 2). The chelipeds are heavily bristled, especially on the dactyli and propodi; not all bristles are shown in the diagrams. Measurements: length of carapace, including rostrum, 21 and 33 mm; rostrum 4.6 and 5 mm; abdomen 33 and 57 mm.

Family CANCRIDAE

Cancer gracilis Dana

Range.—Prince of Wales Island, Alaska, to Playa Maria Bay, Lower California, shallow water to 78 fathoms.

Present collection.—Kyuquot Channel, west coast of Vancouver Island, 74 to 78 fathoms, July 3, 1954.

Remarks.—Previously *C. gracilis* had been reported to a depth of 56 fathoms. Two large male specimens from Nuchatlitz Inlet, Vancouver Island, taken at 26 to 27 fathoms on July 3, 1954, measured 104 and 97 mm in carapace width.

Cancer magister Dana

Range.—Unalaska to Monterey Bay, California, intertidal zone to 98 fathoms.

Present collection.—At four localities from Clayoquot Sound to Quatsino Sound, Vancouver Island, at depths between 58 and 98 fathoms, June 15 to 28, 1954. Queens Sound, 77 fathoms, July 31, 1955; two ♀, 64 and 77 mm.

Remarks.—Rathbun (6) reported that *C. magister* ranged to a depth of 50 fathoms. Phillips (3) recorded it to 93 fathoms, but this reference seems to have been missed by more recent authors.

Acknowledgments

Mr. G. V. Dubokovic collected most of the specimens. Dr. J. F. L. Hart read the manuscript and offered valuable suggestions. The photograph was taken by Mr. C. J. Morley.

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Fig. 3. *Axiopsis spinulicauda.* Dorsal view, female.

THE ANDROGENIC ACTIVITY OF 11-KETOTESTOSTERONE: A STEROID IN SALMON PLASMA[1]

D. R. Idler, P. J. Schmidt, and Jacob Biely[2]

Abstract

The androgenic activity of 11-ketotestosterone, which occurs at a high concentration in the plasma of postspawned male sockeye salmon, has been compared with that of testosterone propionate. The potency of 11-ketotestosterone was 58% as compared with standard testosterone propionate when a chick comb growth assay was employed.

Introduction

11-Ketotestosterone was recently isolated for the first time from the blood of postspawned male sockeye salmon (*Oncorhynchus nerka*) where it occurred at a concentration of 12 μg 100 ml (1). The physiological significance of this compound in salmon is not known. If the steroid could be shown to have androgenic activity, the role that it might play in the sexual maturation of salmon would be worthy of investigation.

Materials and Methods

11-Ketotesterone was synthesized by the bismuthate oxidation of cortisone to produce adrenosterone (2, 3), which was then reduced with $NaBH_4$ (4). Sodium bismuthate (25 g) was added to a solution of cortisone (2.2 g) in 250 ml of 50% aqueous acetic acid. The mixture was shaken for 1 hour at 25° C and centrifuged. The supernatant was diluted with water to 1 liter and extracted with two 1-liter portions of ethyl acetate. The ethyl acetate extracts were washed consecutively with 200 ml of each of 5% sodium carbonate, water, 0.1 *N* acetic acid, and water. The ethyl acetate was flash-evaporated and the residue purified by gradient elution chromatography (5). The adsorbent used was Woelm neutral alumina with grade 3 activity. A 4.5-cm (I.D.) column was packed to a height of 20 cm by adding the adsorbent (ca. 400 g) as a slurry in benzene. The steroid residue was applied to the adsorbent in 10 ml of benzene. The reservoir (12 cm O.D.) contained 2 liters of 10% methanol in benzene. This was siphoned into the mixing bottle (17 cm O.D.), which contained 4 liters of benzene. The flow rate was 10 ml per minute. A steroid (1.37 g = 75%) was obtained between 1100 and 1600 ml which, when chromatographed using Whatman No. 1 paper and heptane:benzene 1:1–70% methanol, had the R_f of authentic U.S.P. adrenosterone. No other ultraviolet-absorbing spots were visible.

[1]Manuscript received October 5, 1960.

Contribution from the Fisheries Research Board of Canada, Chemistry Section of the Technological Station, Vancouver 8, B.C.

[2]Poultry Nutrition Laboratory, Department of Poultry Science, University of British Columbia, Vancouver, B.C.

Can. J. Biochem. Physiol. Vol. 39 (1961)

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Synthetic adrenosterone (1.37 g) was dissolved in 200 ml of 80% *tert*-butanol (6), 0.120 g of $NaBH_4$ was added, and the mixture shaken for ½ hour. Acetic acid was added dropwise until there was no further bubbling, the mixture diluted with 600 ml of water, and the steroid extracted with 3×400 ml of dichloromethane. The dichloromethane was flash-evaporated and the residue subjected to gradient elution with benzene – 10% methanol:benzene using the above described procedure. Fractions were collected and flash-evaporated. The residue, which was assumed to be 11-ketotestosterone, appeared after 1645 ml of solvent was run through the column. The entire residue was contained in a 200-ml portion of solvent. The residue (20 μg) was treated by paper chromatography using the heptane:benzene 1:1–70% MeOH system along with synthetic 11-ketotestosterone previously prepared and characterized (1). There was only one ultraviolet-absorbing substance from the residue and it had the same R_f as authentic 11-ketotestosterone. The yield of 11-ketotestosterone was 0.85 g or 62%. The 11-ketotestosterone used in this study was also purified by paper chromatography (1).

The chick comb weight bio-assay (7, 8) was used to determine the biological activity of the test material relative to that of crystalline U.S.P. testosterone propionate. Each steroid was dispersed in corn oil in four different concentrations. It was found necessary to warm the oil to ensure solution of the 11-ketotestosterone. Equal molar solutions of the two steroids were applied to the combs of single comb White Leghorn male chicks for 8 consecutive days starting with 2-day-old chicks. The total dosage varied from 12 to 64 μg per chick. Each dose had been administered in 0.02 ml of oil to groups of 16 chicks. There were no deaths. The chicks were killed 24 hours after the last dose was administered and the body and comb weights were determined individually. The 12 chicks of each group with the most uniform final body weights were selected for the assay. The four chicks which showed the greatest deviation from the mean body weight were discarded. Corn oil, without any steroid, was also administered to a group of 16 chicks.

Results

The average weights of the chick combs according to the dosage of steroids applied are presented in Table I. The group of chicks that received corn oil without any added steroid had a mean comb weight of 71 mg. These data were subjected to statistical analysis by the method of Irwin (9) as applied by Pugsley (10). The slopes of the log dosage:response curves were found to be 98.9 and 102.6 respectively. The equations of the two regression lines were calculated and the lines are shown in Fig. 1 together with the experimental points. M, the logarithm of the ratio of the activity of the unknown to the activity of the standard, was calculated and the potency of 11-ketotestosterone was found to be 57.6% of that of testosterone propionate. The limits of error were calculated ($p=0.95$) and the range of potency was 46 to 73%.

TABLE I

The response of the chick comb to testosterone propionate and 11-ketotestosterone

Total dosage (mμmoles)	Log of dose	Mean comb weight (mg)	
		Testosterone propionate	11-Ketotestosterone
39.8	1.600	112.7	84.8
79.7	1.901	134.6	112.7
133.1	2.124	159.5	140.5
186.1	2.270	179.6	151.8

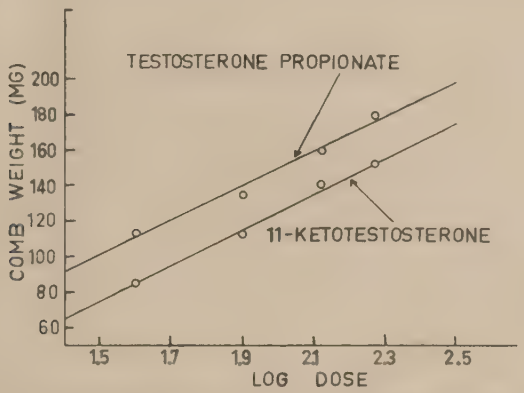

FIG. 1. Linear relationship between the mean comb weight and the logarithm of the dosage of testosterone propionate and 11-ketotesterone.

Discussion

Numerous tests have been used for assessing the androgenic activity of steroids (11). Activity can be determined by administering the steroid either locally or systemically. The comb growth of both capons and chicks has been used as a measure of potency. Other assays, such as the rat seminal vesicle test, have also been used. Different assay procedures often give different potency values. For example, Δ^4-androstene-3,17-dione had 80% of the activity of testosterone by the local capon comb method but had 12% of the activity of testosterone by the systemic capon comb method (11). In another case, it is reported that testosterone has 42% of the activity of androsterone by the local chick comb method (12), while by the local capon comb method it has 179% of the potency of androsterone. These data point out the necessity of defining the assay method and the test animal used when considering potency values.

No reference to the biological activity of 11-ketotestosterone has been found in the literature. Data on some 11- and 17-ketosteroids are available (7). Assuming that androsterone has a potency of 100%, then Δ^4-androstene-3,17-dione is reported to have a potency of 110% by the local chick comb method, while Δ^4-androstene-3,11,17-trione has a potency value of 12%. Thus the

addition of an 11-oxygen to a 17-ketosteroid results in a decrease in potency. This decrease is much greater than that due to the addition of an 11-oxygen to the 17-hydroxylated steroid, testosterone.

Studies now in progress are concerned with the function of this hormone in salmon.

Acknowledgment

The authors wish to acknowledge the technical assistance of Mr. I. I. Bitners of this Station and Miss Genia Biely of the Department of Poultry Science.

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MARINE STEROLS

VIII. IN VIVO TRANSFORMATIONS OF THE STEROL SIDE CHAIN BY A CLAM[1]

U. H. M. Fagerlund and D. R. Idler

Abstract

Analysis of the ozonolysis products of a sterol fraction obtained from clams (*Saxidomus giganteus*) injected with cholesterol-26-C^{14} has demonstrated that these molluscs are able to introduce unsaturation into the cholesterol side chain at C-22 and C-25.

The origin of the sterols of marine invertebrates has for some time been under investigation in this laboratory. A previous publication (1) revealed the ability of a starfish (*Pisaster ochraceus*) to convert injected cholesterol into 7-cholestenol and the ability of two pelecypods, a mussel (*Mytilus californianus*) and a clam (*Saxidomus giganteus*), to synthesize a sterol from injected acetate-2-C^{14}. The exact nature of this sterol was not determined but when the azoyl ester mixture of the sterol fraction was chromatographed, the major portion of the radioactivity was found in a zone which on previous chromatograms of clam steryl azoyl esters has contained monounsaturated Δ^5-sterols. The zone containing 24-methylenecholesterol carried a small amount of radioactivity which, however, constituted only a minor fraction of the total radioactivity in sterols.

In order to ascertain whether the low radioactivity in the 24-methylenecholesterol zone possibly was due to the formation of secondary products, cholesterol-26-C^{14} has been injected into clams. The results demonstrate that the sterol side chain can undergo unsaturation in at least two positions, viz., at C-22 and C-25.

Dehydrogenation in the C-25 position has been observed to occur in mouse and rat liver preparations (2, 3). To the best of our knowledge biological dehydrogenation in the C-22 position of the sterol side chain has not previously been demonstrated experimentally. Whether the reaction is a direct dehydrogenation or a hydroxylation at C-22 or C-23 followed by dehydration is not established by this investigation.

Experimental

Radioactivity was determined with a gas-flow counter equipped with a "micromil" window.

Clams (about 10 cm long) were held in an aerated aquarium filled with sea water and were fed from time to time with macerated brine shrimp. The

[1]Manuscript received October 5, 1960.
Contribution from the Fisheries Research Board of Canada, Technological Station, Chemistry Section, Vancouver 8, B.C.

Can. J. Biochem. Physiol. Vol. 39 (1961)

F.R.B. No. 637

aquarium was chilled from the outside with running tap water. Cholesterol-26-C^{14}* (3.9×10^6 c.p.m.) was dissolved in 0.5 ml of spring salmon oil and the oil was injected at random into the bodies of four clams with a 22-gauge hypodermic needle forced in between the closed shells. Four days after the injection the clam shells were opened and the contents macerated in a Servall Omni-Mixer. The oil was extracted with hot acetone and saponified with potassium hydroxide. The sterol fraction was obtained by chromatography of the nonsaponifiable matter on neutral aluminum oxide "Woelm" (activity grade II).† The portion eluted with 10% methanol in methylene chloride was esterified with *p*-phenylazobenzoyl chloride (4). The mixed esters were chromatographed on silicic acid – Celite 535 using petroleum ether – benzene (5.5:1) to develop the chromatogram (5). Two colored zones were visible on the chromatogram. By its rate of movement the upper zone was identified with zone 2 (or β) of previous chromatograms (6) found to contain 24-methylenecholesterol, and the lower zone identified with the zone containing monounsaturated Δ^5-sterols. Of the total radioactivity located in the two zones, 16.2% was found in the 24-methylenecholesterol zone. The latter zone was eluted, crystallized from benzene–aqueous methanol, and hydrolyzed. A portion of the hydrolyzed material was crystallized from methanol. The melting point was then 122–124°. A second crystallization did not appreciably change the melting point. Although the melting point was low, the infrared spectrum of the crystallized sterol was consistent with that of 24-methylenecholesterol.

Ozonolysis of Upper Zone Acetate

The hydrolyzed material was acetylated and 40 mg were ozonized in a 4:1 mixture of acetic anhydride and acetic acid in an ice–salt bath. The reaction mixture was heated over zinc dust and steam distilled into a sulphuric acid – ethanol–water solution of 2,4-dinitrophenylhydrazine. The contents of the receiver were diluted with water and extracted with ethyl acetate, which was washed with water, dried with sodium sulphate, and evaporated to dryness. The residue was chromatographed on aluminum oxide of activity grade II. The chromatogram was developed with hexane:benzene (2:1). The two zones that appeared had the R_f values of formaldehyde (upper zone) and isovaleraldehyde (lower zone) 2,4-dinitrophenylhydrazones. They were eluted from the column with the same solvent.

Material from the upper zone was chromatographed on Whatman No. 1 filter paper treated with a solution of 5% olive oil in chloroform (7). The chromatogram was developed descending in isopropanol–water (1:1) for 24 hours. The material separated into two spots of approximately equal size. The faster-running substance travelled with the same R_f value as formaldehyde 2,4-dinitrophenylhydrazone. An aliquot (0.75 mg) of the crude upper zone from the aluminum oxide chromatogram was combined with 10.25 mg

*Supplied by Beta Laboratories Inc., Philadelphia, Pa., U.S.A.
†Supplied by Alupharm Chemicals, P.O. Box 755, New Orleans, La., U.S.A.

of non-radioactive formaldehyde 2,4-dinitrophenylhydrazone. The mixture then had a specific activity (S.A.) of 40.9 c.p.m./mg. A constant S.A. of 16.5 c.p.m./mg was attained after three crystallizations from aqueous methanol.

The material from the lower zone was chromatographed on filter paper treated with olive oil. This material also separated into two spots of approximately equal size. The faster-running component had the same R_f value as isovaleraldehyde. The slower-running component travelled at the same rate as methylisopropyl acetaldehyde. This compound has previously been observed in the ozonolysis products from crude preparations of 24-methylenecholesterol from oyster. An aliquot (0.75 mg) of the crude lower zone from the aluminum oxide chromatogram was combined with 10.52 mg of non-radioactive isovaleraldehyde 2,4-dinitrophenylhydrazone. The S.A. of the mixture was 45.5 c.p.m./mg. After three crystallizations the S.A. had dropped to a constant value of 23 c.p.m./mg.

Material recovered from the supernatants from the crystallizations of the mixed isovaleraldehyde 2,4-dinitrophenylhydrazones was dissolved in chloroform. Bromine in chloroform solution was added at room temperature until the solution was red in color. The solvent was evaporated under a stream of nitrogen and the residue crystallized several times from aqueous methanol to a constant activity of 23 c.p.m./mg.

Rearrangement with HCl

A mixture of 40 mg of upper zone acetate and 77.5 mg of non-radioactive 24-methylenecholesteryl acetate, obtained from clams, was dissolved in 5 ml of chloroform and HCl gas was passed through the solution for 8 hours (8). The solution was evaporated with a stream of nitrogen and 5 ml of acetic anhydride was added. The mixture was refluxed for 11 hours. The dehydrohalogenated product was then ozonized in an ice–salt bath. Zinc dust was added and the mixture was steam distilled into a 2,4-dinitrophenylhydrazine solution as outlined above. The evaporated ethyl acetate extract was chromatographed on aluminum oxide. Three zones developed on the chromatogram. The mobility of the bottom zone was similar to that of the 2,4-dinitrophenylhydrazone of 3-methyl-2-butanone and isovaleraldehyde. One milligram of the material obtained from the bottom zone with a radioactivity of 78 c.p.m. was combined with 8.8 mg of non-radioactive authentic 3-methyl-2-butanone 2,4-dinitrophenylhydrazone and crystallized from aqueous methanol. In two crystallizations the mixture apparently lost all its radioactivity. The material obtained from the bottom zone was then crystallized undiluted. Three crystallizations removed most of its radioactivity. However, four additional crystallizations failed to remove the last traces (1 to 2 c.p.m. mg over background) of radioactivity. When isovaleraldehyde 2,4-dinitrophenylhydrazone was added to material obtained from the supernatant from the first crystallization, a 2,4-dinitrophenylhydrazone was obtained that could be crystallized to constant activity.

Discussion

Cholesterol-26-C^{14} has been injected at random into clams (*Saxidomus giganteus*). Ozonolysis of the crude 24-methylenecholesterol, obtained by chromatography of the azoyl esters of the sterol fraction, resulted in the formation of a mixture of steam volatile carbonyl compounds, from which radioactive isovaleraldehyde and formaldehyde could be isolated as 2,4-dinitrophenylhydrazones. This demonstrates the introduction of both a C-22 and a C-25 double bond into the sterol molecule. Both conversions were observed in the order of 1% calculated on the total radioactivity in the sterol fraction. The fact that dehydrogenation can take place at both the C-22 and the C-25 positions in the same invertebrate raises the possibility that both double bonds can be introduced into the same molecule, with the formation of a C-22,25 diunsaturated side chain. Such a side chain configuration has been observed in a sterol isolated from cucurbit seeds (9). The ozonolysis of such a sterol could conceivably produce the unsaturated aldehyde 3-methyl-3-butenal. To exclude the possibility that this aldehyde contaminated isovaleraldehyde, the 2,4-dinitrophenylhydrazone of the mixture of the radioactive and non-radioactive isovaleraldehyde was treated with bromine and recrystallized several times. No change in the S.A. was observed.

The addition of hydrogen chloride to 24-methylenecholesteryl acetate and subsequent dehydrohalogenation with acetic anhydride result in migration of the C-24(28) double bond to the C-23 position. Ozonolysis then affords 3-methyl-2-butanone. The isolated ketone was found to be free of any significant amount of radioactivity.

The presence in clams of a system capable of dehydrogenation in the C-22 and C-25 positions and the earlier reported C-22,25 diunsaturated sterol isolated from other biological material are consistent with a theory advanced by Tschesche and Korte (10) as to the mode of formation of C-28 and C-29 sterols in plants. According to this theory the activation of C-24 by a system of C-22 and C-25 double bonds would facilitate the addition of one or two carbon-units at C-24. An intermediate with three double bonds would first be formed and by hydrogenation would give rise to C-28 and C-29 sterols. If the assumption is made that the same reaction sequence takes place in clams, 24-methylenecholesterol would be derived from a precursor with a cholesterol-type side chain. 24-Methylenecholesterol would then have a lower specific activity than the Δ^{22} and Δ^{25} precursors. Furthermore, consideration must be given to the possibility that 24-methylenecholesterol has a specific function in the cell. A differentiation in the function of sterols has been indicated in certain insects (11). If 24-methylenecholesterol has a structural role, the turnover of the sterol can be expected to be slight because of the slow growth rate of the clam. Consequently the addition of C-28 may take place at a comparatively slow rate. The final activity of 24-methylenecholesterol might then be too small to detect under the present conditions. This reasoning is consistent with the observation that the 3-methyl-2-butanone obtained from

the side chain through hydrogen chloride rearrangement and ozonolysis, when crystallized, initially suffered a sharp drop in S.A., but could not be entirely freed from radioactivity.

It has been reported in a previous publication (1) that the injection of radioactive acetate into clams resulted in only negligible radioactivity in the 24-methylenecholesterol zone. For the same reasons as outlined above, it cannot be concluded from those experiments that 24-methylenecholesterol is not synthesized from acetate.

Acknowledgment

We are indebted to Dr. D. B. Quayle, Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C., for supplying the clams used in this investigation.

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MAGNESIUM AS A FACTOR IN DETERMINING THE COMPARATIVE SENSITIVITY OF MARINE BACTERIA TO FOUR TETRACYCLINE ANTIBIOTICS

B. A. Southcott and H. L. A. Tarr

During a study of the keeping quality of Pacific coast dogfish (*Squalus suckleyi*) (1, 2) typical bacterial colonies were isolated from a number of plates, and cultures of these isolates were studied by the usual procedures in order to permit a rough classification. When the sensitivity of each culture to four of the tetracycline antibiotics was determined, using sensitivity disks (Bacto, 10.0 μg), it was found that they were less sensitive to the tetracyclines on sea water agar plates containing peptone, yeast extract, $FePO_4.2H_2O$, and artificial sea water (1) than on nutrient agar plates (Difco). It was also observed that the sensitivity to the tetracyclines differed considerably on the two agars and that sensitivity to OTC (oxytetracycline) was invariably lower than that to CTC (chlortetracycline), TC (tetracycline), or DCTC (demethylchlortetracycline). Typical results with five different cultures are given in Table I.

TABLE I

Effect of agar media on inhibitory effect of tetracyclines

Culture	Diameter of inhibitory zone (mm)							
	Nutrient agar				Sea water agar			
	CTC	OTC	TC	DCTC	CTC	OTC	TC	DCTC
1 Gram+ coccus	29	25	29	29	23	9	18	21
5 Gram+ coccus	28	21	25	32	0	0	0	0
51 Gram− rod	12	0	11	11	0	0	0	0
79 Gram+ rod	44	35	41	40	23	0	18	19
109 Gram+ coccus	29	24	26	27	16	0	12	12

In an attempt to identify the substance or substances responsible for the apparent resistance of these cultures to the tetracyclines in sea water agar, a synthetic agar[1] was prepared to which various inorganic salts were added. Tests with typical organisms revealed that, with a single exception, under the experimental conditions, Mg^{++} but not Na^{+} or Fe^{+++} contributed to the increase in resistance to tetracyclines using the synthetic agar. The results obtained using an orange-pigmented Gram-positive coccus isolate (culture 109, Table I) are given in Table II.

Culture 5 (Table I) became completely unaffected by the tetracyclines where sufficient Mg^{++} was added to the medium, the amount of Mg^{++} required to exert this depending on the tetracycline present (Table III).

[1]Composition of synthetic agar per 100 ml: 0.3 g glucose; .09 g $(NH_4)_2SO_4$; .013 g $(NH_4)_2HPO_4$; 1.5 g agar; 100.0 μg each of riboflavin, Ca pantothenate, thiamine HCl, and niacin; 20.0 μg each of pyridoxal hydrochloride and para-aminobenzoic acid; 1.0 μg each of biotin, folic acid, and vitamin B_{12}; 40.0 mg alanine; 20.0 mg glutamic acid; 16.0 mg aspartic acid; 8.0 mg each of arginine hydrochloride, lysine hydrochloride, isoleucine, methionine, phenylalanine, threonine, valine, tryptophane, and serine; 4.0 mg each of histidine, leucine, proline, tyrosine, cystine, and glycine.

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These results would seem to afford a possible explanation for the fact that CTC has proved superior to both OTC and TC for fish preservation (3). Fish muscle contains 0.01 to 0.03 *M* Mg^{++} (4). So far, the possible inhibitory effect of Ca^{++}, which occurs in somewhat higher concentrations in fish muscle, has not been explored. The chelating effect of the tetracyclines for divalent ions has frequently been described (5, 6), but results similar to the above do not appear to have been reported. Shellfish such as crab, oysters, and shrimp require higher concentrations of tetracycline antibiotics for successful preservation than do fish (3), and a possible explanation of this could be that shellfish normally have considerably higher concentrations of both Mg^{++} and Ca^{++} ions.

TABLE II

Influence of added salts on inhibitory effect of tetracyclines

Salts added (molar concentration)			Diameter of inhibitory zone (mm)			
			CTC	OTC	TC	DCTC
NaCl	$MgCl_2$	$FePO_4$				
			54	23	40	40
0.1			54	23	40	40
0.2			50	24	40	40
	0.05		36	14	23	32
	0.1		32	14	18	26
		0.00006	50	22	43	43
		0.00012	49	23	40	45
0.2	0.1		36	17	17	29
0.2		0.00012	44	22	42	44
	0.1	0.00012	33	13	19	25
0.2	0.1	0.00012	30	16	18	25

TABLE III

Effect of Mg^{++} concentration on inhibitory effect of tetracyclines

$MgCl_2$ (molar concentration)	Diameter of inhibitory zone (mm)			
	CTC	OTC	TC	DCTC
0	29	11	26	29
0.005	22	9	16	27
0.01	20	9	16	25
0.03	19	Trace	13	21
0.05	17	0	9	21
0.08	17	0	Trace	18
0.1	13	0	0	17
0.3	0	0	0	12
0.5	0	0	0	0

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RECEIVED OCTOBER 31, 1960.
FISHERIES RESEARCH BOARD OF CANADA,
TECHNOLOGICAL STATION,
VANCOUVER 8, B.C.

Reprinted from ANALYTICAL CHEMISTRY, Vol. 32, Page 1670, November 1960
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Colorimetric Determination of Halogenated Nitrophenols Added to Streams as Sea Lamprey Larvicides

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▶ In the application of the specific sea lamprey larvicide, 3-trifluoromethyl-4-nitrophenol (TFM), to certain tributaries of the upper Great Lakes, there is a range of concentration within which all larvae are killed without harm to most other forms of aquatic life. A rapid procedure for determining TFM under field conditions is based on measurement of the intensity of the alkaline color. Variations in the response of the colorimeter caused by changes in stream color are corrected by the use of a nomograph to convert instrumental readings to parts per million of TFM.

A RECENT DISCOVERY of several halogenated nitrophenols which are selectively toxic to sea lampreys (*1*) has resulted in a program of chemical control aimed at eradicating this destructive parasite from the upper Great Lakes. Sea lampreys spend their initial years in larval form in the beds of certain Great Lakes tributaries and at this stage are most vulnerable to attack by selective toxicants (*2*).

The most effective of these toxins to date is 3-trifluoromethyl-4-nitrophenol (TFM, α,α,α - trifluoromethyl - 4 - nitro-*m*-cresol). TFM is utilised in the treatment of streams as a liquid formulation of the more soluble sodium salt of the phenol combined with suitable nontoxic solvents. This compound, like the others of the group, becomes toxic to other forms of aquatic life if its concentration in the water rises above a certain level. The differential toxic effects of TFM vary from stream to stream. In all cases encountered to date, concentrations of 0.5 to 7 p.p.m. of the larvicide in the water have been completely effective.

A colorimetric method has been developed which permits the rapid

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determination of the larvicide concentration at any point in the treated water mass as it progresses downstream. The method is based upon developing the full, yellow alkaline color of the halogen nitrophenol and analysis of the resulting solution in a colorimeter (*3*). Analytical techniques have been adapted to meet the various problems presented by the particular natural waters in which the nitrophenol is being introduced.

Difficulties due to solid suspended matter have been eliminated by filtering the sample through a Millipore filter and buffering the filtrate at pH 9 with borate. The filtering procedure effectively removes interfering clay suspensions and the borate buffer minimizes the formation of precipitates.

Had the natural color of a stream remained constant during an application of larvicide, this color would have presented no problem. It would have sufficed to adjust the instrument to read zero on the stream water and to construct a standard curve, the standard being made up with the same water. This was the procedure originally employed.

Field experience, however, soon pointed to the need for a procedure for determining the amount of TFM in a given sample when the background color of the water was unknown. This situation arose during treatments at downstream sampling stations when local storms suddenly added unknown amounts of surface water to the stream. The resultant alteration of background color could hardly be determined. Conversely, the subsidence of a stream during a period of treatment was also accompanied by changes in background color. In addition, uncertainties arose below the confluence of two tributaries having differing natural water colors. The color of the water below such a junction changed unpredictably as the volume of flow of the contributing streams varied independently.

Because nitrophenols are practically colorless in acid solution, the color in any acidified sample containing TFM is due to the natural water itself. Although this color is significantly less than the color of the natural water at high pH's, the ratio of these two color intensities is almost constant. This ratio, established as a constant, and an optical measurement of an acidified sample of treated water, together permit calculation of the amount of background color in the buffered sample and adjustment of the colorimeter so that its reading measures the amount of TFM present.

Under conditions of unchanging natural stream color, the concentration of TFM in the treated water could be determined from a standard curve derived from colorimetric measurements of standard solutions of TFM prepared in the untreated water. However, when the background color changed, the form of the standard curve also changed. Hence, a family of standard curves would be required to anticipate changes in background color during treatment. A nomograph was constructed which eliminated the necessity of preparing a series of standard curves and avoided the difficulty of interpolating between them.

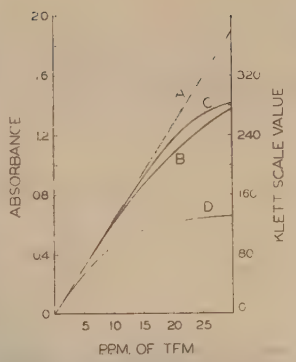

Figure 1. Standard curves for TFM in 1% sodium hydroxide

- *A.* Beckman Model DU spectrophotometer
  - λ = 395 mμ
  - 1-cm. Corex cells
  - Tungsten lamp
- *B.* Klett-Summerson colorimeter
  - No. 42 filter
  - 1-cm. light path with 1 × 4 cm. cell
- *C.* Bausch & Lomb Spectronic 20 spectrophotometer
  - λ = 395 mμ
  - 1/2-inch cuvette
- *D.* Coleman Model 14 spectrophotometer
  - λ = 395 mμ
  - 19-mm. cuvette

INSTRUMENTATION

TFM is a fairly strong acid (pK 6.07 ± 0.03), whose full alkaline color is developed at pH 8.0 and above (*3*). Under these conditions it has a molar absorbance of 13,130 at 395 mμ and in a precise instrument such as a Beckman Model DU spectrophotometer it follows Beer's law (Figure 1, *A*). Because of the broad band of wave lengths (400 to 450 mμ) transmitted by the No. 42 Klett-Summerson filter, however, the absorbance of TFM solutions measured with the Klett-Summerson colorimeter appears to deviate from Beer's law (Figure 1, *B*). The Bausch & Lomb Spectronic 20 spectrophotometer (set at 395 mμ, band width 20 mμ) gives readings corresponding to Beer's law up to about 15 p.p.m. of TFM (Figure 1, *C*). The Coleman Model 14 spectrophotometer (set at 395 mμ, band width 35 mμ) did not yield readings consistent with Beer's law (Figure 1, *D*).

The mechanical complexity and sensitivity of the Beckman Model DU spectrophotometer prohibited field use. The Coleman Model 14 spectrophotometer was rejected for the same reason, as well as the fact that its optical characteristics were unsatisfactory. The Bausch & Lomb Spectronic 20 was also unsatisfactory. Although this instrument has desirable optical characteristics within the range of concentrations normally applied in streams, it does not operate satisfactorily from the portable a.c. power sources available in the field. Operation of the instrument by storage batteries and a Terado converter was also unsatisfactory under field conditions.

The Klett-Summerson colorimeter, in spite of its less desirable optical characteristics, was therefore selected for field use because of its mechanical ruggedness and its satisfactory performance with the power sources available at stream treatment sites.

PROCEDURE

The following colorimetric procedure yields reliable information on the concentration of TFM in a natural water, whether the natural color of the stream is known or not. While the procedure has been developed specifically for TFM, the method should be applicable to any of the other larvicides. The appropriate physical properties of these compounds, as well as those of TFM, have been described in detail (*3*).

Apparatus and Reagents. All measurements are made with a Klett-Summerson photoelectric colorimeter with a No. 42 filter (400 to 450 mμ) and 2 × 4 cm. rectangular cell, utilizing the 4-cm. light path. Values recorded are those read directly from the dial of the instrument. Conventional laboratory ware and medium speed filter paper are used for the removal of coarse particles in samples. A vacuum-operated Millipore Filter (Model XX60, Millipore Filter Corp., Watertown, Mass.) with Type HA Millipore filter paper (pore size 0.45 ± 0.02 micron) is used for the removal of very fine particles. A Beckman portable pH meter has been used successfully in the field, and is indicated for measurements that the analyst at the site of a treatment deems necessary. All pH determinations given in this report were made with a Beckman Zeromatic pH meter.

All chemicals mentioned below are reagent grade unless otherwise specified. 3-Trifluoromethyl-4-nitrophenol (TFM), technical grade, was recrystallized from benzene (m.p. 75–6° C.). Borate buffer, a saturated solution of sodium tetraborate, $Na_2B_4O_7 \cdot 10H_2O$, in water at

25° C. was prepared from distilled water and excess salt. Stock solution of TFM (500 p.p.m.) was prepared by dissolving exactly 500 mg. of pure TFM in 10 ml. of 1% NaOH. After the solid is completely dissolved, the solution is diluted to 1 liter.

Standard Solutions of TFM. At least two standard solutions containing 1 to 9 p.p.m. of TFM are prepared by diluting appropriate volumes of the 500-p.p.m. stock solution with stream water. This water must have been filtered, first through medium speed filter paper to remove coarse particles, then through the Millipore filter to remove fine suspensions. During the preparation of each standard, sufficient borate buffer is added to ensure a pH of 9.0 ± 0.1. In general, 2 ml. of buffer per liter of solution is sufficient. The Klett scale values of the standards are measured with the colorimeter adjusted to give a zero reading against a filtered stream water blank, buffered with borate. These standards are used to adjust the nomograph as described subsequently.

Determination of Basic-Acidic (B/A) Ratio of Background Color. A sample of about 500 ml. of filtered stream water is divided into two portions. The first is buffered as above, and to the second portion is added 10% hydrochloric acid until the pH is less than 3.0. The Klett scale values of these portions are determined with the colorimeter adjusted to give a zero reading on distilled water. The ratio of the scale reading obtained with the buffered sample to that obtained with the acidic sample is then calculated. This figure is referred to as the B/A ratio.

Calibration of Nomograph. The nomograph consists of three scales (Figure 2). Scale *A* is the Klett scale value of the buffered stream sample containing TFM. Scale *B* refers to the concentration of TFM in this sample in parts per million. Scale *C* represents the Klett scale value of the buffered stream blank which has either been measured directly or calculated from the B/A ratio.

The nomograph is adjusted to a particular stream water before introduction of TFM to the stream. The standard solutions, prepared as described, are used. The Klett scale value of the buffered stream blank is aligned with the known concentrations and scale values of the standards. The Klett scale value and the concentration of one of the standards are located on scales *A* and *B* of the nomograph, respectively. These two points are connected by a straight line which is extended over the movable *C* scale. The *C* scale is then moved along its axis until the known Klett scale value of the buffered stream blank is under this straight line. The Klett scale value of the remaining standard solution is used to check the nomograph setting. The position of the *C* scale is then secured.

Determination of TFM in Water Samples. Stream water samples containing TFM are collected in 500-ml. polyethylene bottles. After preliminary filtration, about 50 ml. (enough to rinse and fill the optical cell) is filtered through the Millipore filter and buffered with 5 to 10 drops of borate solution. When a sample of water is received, the procedure is routine if the background color is known. The Klett scale value is determined with the instrument adjusted to read zero on a solution having the same color as the background.

If the background color is unknown, the filtered sample is divided into two portions, one of which is buffered with borate solution, while the other is acidified to a pH of less than 3.0 with 10% hydrochloric acid. With the colorimeter zeroed on distilled water, the Klett scale value of the acidified sample is determined. This value is multiplied by the B/A ratio; the resulting product represents the Klett scale value of the buffered sample if no TFM were present. The dial of the colorimeter, zeroed on distilled water, is set at this calculated background value and a solution is prepared in the optical cell which zeroes the galvanometer needle with this dial setting. This can be done, if the stream is too dark, by putting a sample of untreated stream water in the cell and adding distilled water, or, if the stream is too light, by employing a darker solution such as sodium chromate. The colorimeter is then adjusted to read zero on this solution. After this adjustment, the Klett scale value of the borate-buffered stream sample containing TFM is determined.

From the known Klett scale values of the buffered stream background color and of a buffered sample containing TFM, the corresponding concentration of TFM can be found by use of the nomograph. To determine the concentration of TFM in a sample of stream water, the Klett scale value of the buffered stream blank is located on scale *C* and the Klett scale value of the buffered stream sample containing TFM is located on scale *A*. A straight line joining these points (in practice either a straight edge or a stretched thread) will intersect the concentration scale (*B*) at a point representing parts per million of TFM present.

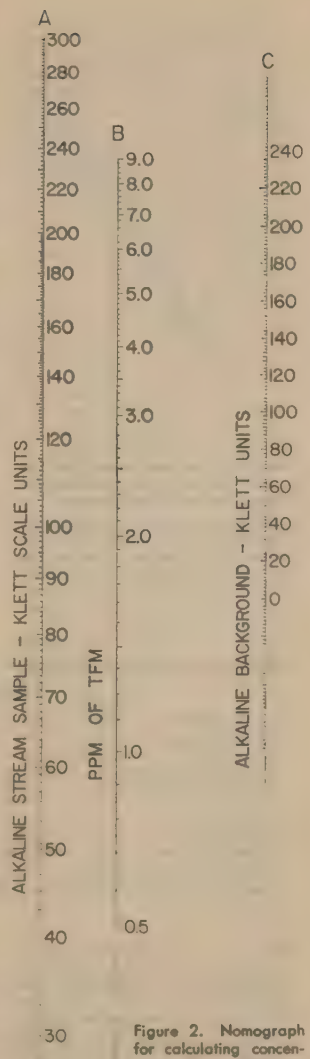

Figure 2. Nomograph for calculating concentration of 3-trifluoromethyl-4-nitrophenol in stream water with Klett-Summerson colorimeter

EXPERIMENTAL

Prevention of Precipitates in Alkaline Stream Water Samples. Earlier investigations had shown that a pH of at least 8 was necessary to develop the full, alkaline color of the TFM (*3*). Good practice dictated that a pH of about 9 be established in all analytical solutions. However, where pH's above 9 were created in natural waters by the addition of excess alkali, suspensions of insoluble carbonates were likely to form. At first, this was controlled by cautiously adding sodium hydroxide to stream water samples until the pH was between 8.5 and 9.0. This procedure proved impractical in the field, and a buffer was sought which would provide the desired pH level.

A saturated solution of sodium tetraborate was satisfactory; the amount required was always less than 10 ml. per liter and in practice about 2 ml. per liter of treated stream water. The precise quantity to be added must be determined for each stream water, as it will vary with the hardness of the water. The pH of the buffer itself is less than 9.2.

The problem of carbonate precipitates arose also in the preparation of standard solutions of TFM in stream water. Originally, the stock solution of 500 p.p.m. of TFM was also prepared in 1% NaOH. This ensured the conversion of all of the TFM to its more soluble sodium salt (the form of the phenol utilized in actual stream applications) and the development of full nitrophenol color. The pH of standards prepared from this stock solution was usually above 9. This produced turbidity in hard waters. It was not possible to reduce this alkalinity by the addition of borate buffer. These difficulties were not encountered, however, when the stock solution was prepared so that the final sodium hydroxide concentration was only 0.01%. The subsequent addition of buffer to the standards produced a suitable pH, while the same time sufficient caustic was present to convert the TFM to its sodium salt.

The efficacy of this procedure is illustrated by optical measurements of two series of standard solutions which were prepared with water from the Trout River, Presque Isle County, Michigan (Table I). Series A standards were prepared from a 500-p.p.m. stock solution of TFM in 1% NaOH. Series B standards were prepared from a 500-p.p.m. stock solution of TFM in 0.01% NaOH with borate buffer added to each standard at the rate of 2 ml. per liter. The higher Klett scale values observed for the series A standards are due to precipitates produced at the high pH levels resulting from excess caustic in the solutions. Erratic readings in the more highly concentrated solutions of this series are due to differential settling of turbidities formed.

Removal of Clay Suspensions from Stream Samples. Many streams contain suspensions of clay which were not removed by ordinary methods of filtration. The high absorbance of these very fine suspensions remaining in the filtrates reduced instrumental accuracy, while their tendency to settle out at different rates caused variations in background color determinations.

When stream waters containing clay suspensions were passed through Millipore filter paper, rated to remove particles down to 0.45 micron, practically all turbidity was removed. The data in Table II illustrate how Millipore filtration removed suspended matter from waters of the Firesteel River, Ontonagon County, Michigan, and the Stokely River, Ontario. The Firesteel River is a heavily silted stream, whereas the Stokely River appears visually "clear." Turbidity measurements were made with a Hellige Turbidometer, No. 6035, calibrated for the range between 0.5 and 1 p.p.m. of silica as well as higher concentrations.

Both turbidity and absorbance measurements of the filtrates clearly demonstrate the efficiency of the technique. The residual Klett scale values for the Millipore-filtered samples represent only the natural color of the stream water. Many seemingly clear streams, such as the Stokely River, apparently contain enough subvisible suspended matter to affect any measurements made with the Klett-Summerson colorimeter.

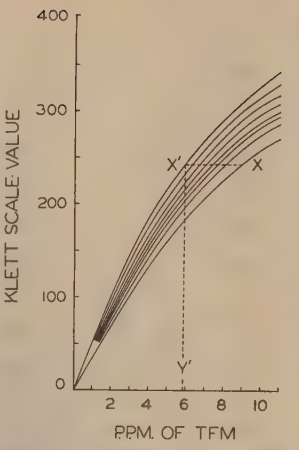

Figure 3. Standard curves for TFM in distilled water and in mixtures of distilled and Milligan Creek water

Uppermost standard curve, TFM in distilled water. Moving downward, successive curves represent TFM in mixtures of Milligan Creek water and 90, 80, 70, 60, 50, 30, and 10% of distilled water

Determination of Background Color in the Presence of TFM. A procedure has been described for determining the natural color of a stream when it contains TFM. The validity of this procedure is based on two premises: that the entire Klett scale value for the sample after acidification is due to natural coloring matter in the water, and that for the TFM-free stream water, the ratio (B/A ratio)

Table I. Klett-Summerson Scale Readings of Standard Solutions of TFM

[Prepared with Trout River water from 500-p.p.m. stock solutions of TFM in (Series A) 1% NaOH and (Series B) in 0.01% NaOH with borate buffer added]

Concn. of TFM, P.P.M.	Series A		Series B	
	pH	Klett scale value	pH	Klett scale value
3	9.5	272	8.95	133
5	9.9	390	8.95	193
7	10.4	430	8.95	246
9	10.7	530	8.95	285
11	11.0	495	8.95	320
13	11.2	540	8.95	350

Table II. Removal of Suspended Matter from Firesteel and Stokely River Waters

Treatment	Turbidity Measurements				Klett Scale Value
	Tube, mm.	Filter	Reading	P.p.m. as SiO_2	
Firesteel River water					
Unfiltered	20	None	32	150	478
1st ordinary filtration	20	None	11.5	12.0	102
2nd ordinary filtration	50	Light	33.5	9.8	92
Millipore filtration	50	Dark	17	0.47	64.5
Stokely River water					
Unfiltered	20	None	25	44.5	56
1st ordinary filtration	50	Light	39	12.5	41
Millipore filtration	50	Dark	12	0.18	14

Table III. Residual Acid Color of TFM Solutions

Concn. of TFM, P.P.M.	Before Acidification		After Acidification	
	pH	Klett scale value	pH	Klett scale value
A. Solutions prepared from 500-p.p.m. stock solution in 0.01% NaOH and distilled water				
1	6.10	28	2.85	0
5	6.30	162	2.70	3
10	6.90	301	2.85	5
B. Solutions prepared from liquid formulation of TFM and distilled water				
1	4.90	7	2.75	0
5	5.00	41	2.80	4
10	5.20	108	2.70	7

$$\frac{\text{Klett scale value at pH 9}}{\text{Klett scale value at pH 3}}$$

is a constant.

The first premise is reasonable, since the yellow color of nitrophenols is largely discharged by acidification. This was true for TFM standards prepared as described earlier (no buffer added) as well as for aqueous solutions of TFM prepared from one of the commercial liquid formulations currently used in field applications (Table III).

The second premise, that the B/A ratio is a constant, has been demonstrated in several ways. In one experiment, samples of water from Milligan Creek, Presque Isle County, Michigan, were diluted with distilled water and the B/A ratio was derived for each dilution by determining the Klett scale value of acidified and borate-buffered portions. Dilution of a highly colored stream water with a clear dilutant did not affect the B/A ratios; these ratios of diluted samples differed neither from each other nor from that of an undiluted sample of the water in significant degree (Table IV).

In another experiment, waters from streams in the same area having similar B/A ratios but differing color intensities were mixed. Water from Milligan Creek (B/A ratio 1.35) was added to Carp Creek water (B/A ratio 1.36), so that several stages of color intensification of the latter were achieved. The B/A ratios of these mixtures were almost identical with those ratios of the original stream waters (Table V).

The reliability of the assumption that neither dilution nor intensification of the natural color of streams would significantly alter the B/A ratio was further demonstrated under natural conditions by noting changes in this property in periodic water samples taken from a single stream. Water from Carp Creek was collected and examined twice weekly between June 8 and October 15, 1959. In this period, the B/A ratio of the stream water samples had a mean value of 1.37, a range from 1.29 to 1.45, and a standard deviation of ±0.035.

Significant changes in the B/A ratio during the whole period were gradual and tended to reflect broad seasonal variations in precipitation and water level. Short-term fluctuations—i.e., during monthly periods—were, however, so small as to lack significance. Because the duration of a single stream treatment seldom exceeds several days, long-term changes in the B/A ratio are of no practical concern.

A number of streams covering a broad area also were examined to determine the extent of geographic variation of the B/A ratio. Single samples were collected from nine tributaries of eastern Lake Superior and seven tributaries of northern Lake Huron. Lake Superior tributaries were: Big Carp, Cranberry, Goulais, Stokely, Harmony, Chipewa, Batchewana, Sable, and Pancake Rivers, District of Algoma, Ontario. Lake Huron tributaries were: Trout, Schwartz, Carp, and Milligan Creeks and the Ocqueoc River, Presque Isle County, Michigan; and Green and Little Billies Creeks, Cheboygan County, Michigan.

The B/A ratios of waters collected from these streams had a mean value of 1.37, a range from 1.30 to 1.46, and a standard deviation of ± 0.045. The range of values observed for these widely scattered tributaries corresponded very closely to that range recorded for periodic samples from a single stream of the group during a 4-month period. This ratio, therefore, shows little variation from watershed to watershed within rather broad geographic areas in the Great Lakes basin.

The Nomograph. During exploration of the characteristics of the B/A ratio, several series of unknown concentrations of TFM were prepared in various dilutions of stream waters. When the Klett-Summerson colorimeter had been adjusted to read zero on the appropriate blank, the concentration of TFM in these unknowns was determined by converting the Klett scale value to parts per million from a single calibration curve. However, a change in the natural stream color not only invalidated the calibration of the colorimeter, but also altered the curvature of the standard curve (Klett scale value *vs.* concentration), even when the colorimeter had been readjusted to read zero on a sample of water having the new background color. This latter effect, due probably to the optical characteristics of the instrument, is illustrated in Figure 3, which shows a series of curves derived from standard solutions of TFM in various mixtures of distilled and Milligan Creek water. All standard solutions were prepared in Millipore-filtered and borate-buffered dilutions of creek water, and the colorimeter was adjusted to read zero on the appropriate mixture from which the standards were prepared. The greater the color intensity of the blank, the lower the slope of the curve, so that the Klett scale values for a given concentration of TFM decrease with increasing blank values.

Furthermore, a standard curve prepared with the water of one stream may have a curvature different from that of a curve prepared with water from another stream having an identical background color. It cannot be assumed, then, that water samples from any two streams containing equal concentrations of TFM will yield the same Klett scale values. Thus, to anticipate not only possible changes in background but also unique background characteristics, it would be necessary to provide a family of standard curves for every stream, prepared from a number of dilutions of the natural water. These data would not only be tedious to prepare in the field but difficult to use because of the necessity of interpolating between curves.

The foregoing considerations illustrate that a third variable—viz., stream background value—must be introduced into the determination of TFM concentration. By replacing the customary graphical representation of the standard curve with a nomograph, the

Table IV. Effect of Dilution on B/A Ratio for Milligan Creek Water

Sample	pH			Klett Scale Value			B/A Ratio
	Initial	Acidic	Basic	Initial	Acidic	Basic	
Undiluted Milligan creek water	7.70	2.90	8.95	242	190	257	1.35
Diluted with distilled water							
20%	7.65	2.85	8.95	199	156	210	1.34
40%	7.60	2.80	8.95	150	119	162	1.36
75%	7.30	2.90	8.95	66	54	73	1.35

Table V. Effect on B/A Ratio for Carp Creek Water of Intensification of Color by Addition of Milligan Creek Water

(Carp Creek water, B/A ratio 1.36; Milligan Creek water, B/A ratio 1.35)

% Milligan Creek Water in Carp Creek Water	pH			Klett Scale Value			B/A Ratio
	Initial	Acidic	Basic	Initial	Acidic	Basic	
0	8.10	2.95	8.95	82	63	86	1.36
10	8.10	2.80	8.95	100	77	105	1.36
20	8.10	2.95	8.95	116	89	120	1.35
30	8.10	2.75	8.95	131	101	138	1.37
40	8.05	2.70	8.95	150	115	156	1.35
50	8.05	2.90	8.95	165	127	171	1.35

analytical technique was made more versatile without sacrificing simplicity. To minimize the effect of instrumental variation and to make possible interpolation between observational data, the nomograph shown in Figure 2 was evolved; it was based on values obtained from the family of curves represented in Figure 3.

The nomograph was constructed in the following manner.

Three parallel straight lines were drawn, representing in order from left to right, (*A*) the Klett scale value for the buffered stream sample containing TFM, (*B*) the concentration of TFM in parts per million, and (*C*) the Klett scale value for the buffered, TFM-free stream water.

The axis, *C*, representing Klett scale values of the borate-buffered stream blanks, was marked off in arithmetic intervals. A point on the sample scale, *A*, was chosen somewhat arbitrarily and assigned a value representing the Klett scale reading of the 9-p.p.m. solution of TFM in distilled water. The zero point of the background scale, *C*, represented the Klett scale value of distilled water. A line was drawn joining these two points which intersected the concentration, *B*, at a point corresponding to 9 p.p.m. This latter point was so designated. The seven Klett scale background values corresponding to the seven mixtures of creek and distilled water next were located as points on the background scale, *C*. Straight lines were drawn from each of these seven points through the 9 p.p.m. point on the *B* scale, making seven more intersections on the sample scale, *A*. The eight points on the *A* scale were then assigned appropriate Klett scale values for the eight 9-p.p.m. standards which were obtained from the corresponding standard curves (Figure 3).

The process was then reversed. The Klett scale value of the 9-p.p.m. standard in the darkest water was equal to that of a reading for a lower concentration of TFM in distilled water (see $X - X'$, Figure 3). A line was drawn on the nomograph joining the zero point of the *C* scale to the lowermost of the points on the *A* scale (Klett scale value of the 9-p.p.m. standard in the darkest water). The intersection of this line with the concentration scale, *B*, corresponded to the concentration of a hypothetical solution of TFM in distilled water. This concentration was determined from the standard curve for distilled water (see $X' - Y'$ in Figure 3) and was so designated on the *B* scale. This point on the *B* scale in turn gave rise to eight more points on the sample scale, *A*. This was accomplished by connecting the points on the *C* scale used previously with the new value located on the concentration scale, *B*, and extending these lines to intersect the sample scale, *A*. This process was repeated, the concentration scale was extended downward, and for each interval of concentration a segment of the *A* scale was constructed. Thus, both of the unknown scales were extended until suitable ranges of concentrations and Klett scale values were obtained.

This process resulted in irregular gaps in scales *A* and *B*. These were filled in by constructing smooth curves relating linear distance on the scale with scale interval. By interpolating from these curves, desirable scale intervals were laid off.

In practice, the *C* scale is movable, so that the nomograph may be adapted to different waters. To set the nomograph for a particular stream, a standard solution of TFM is prepared in the water to be treated, and calibration is carried out as described under "Procedures."

Determination of TFM in Unknown Samples. An experiment was conducted to test the accuracy of the entire procedure for the determination of TFM in stream water samples having unknown background values. These tests simulated conditions which could normally arise during a field application of the larvicide.

Samples of water were obtained from Carp and Milligan Creeks, Presque Isle County, Michigan, and the Stokely and Kaministikwia Rivers, Ontario. A portion of each sample was diluted with an equal volume of distilled water, and, using these mixtures, standard solutions of TFM were prepared. For each hypothetical stream treatment, this diluted stream water represented the conditions prevailing at the start of the application and was used in each instance to determine the B/A ratio and adjust the colorimeter. The standards were used to set the nomograph, as described earlier.

Three unknown solutions of TFM were made up for each stream. One of these unknowns utilized stream water diluted with an equal volume of distilled water, a second was made up with undiluted stream water, and the third was prepared with stream water diluted with twice its volume of distilled water. Thus one unknown was in darker water, one in lighter water, and one in water of the same color intensity as that used for the standards.

Each of the unknowns was treated in the same way. Using B/A ratios previously determined, the buffered background values of the unknowns were calculated and the Klett scale values were determined. The nomograph, appropriately adjusted for each stream, was then used to convert these values to parts per million of TFM in the unknowns.

Determinations of concentrations of TFM in these unknowns were never more than 0.2 p.p.m. at variance with the actual values. The average error for 12 determinations was 0.1 (Table VI).

Table VI. Determination of Unknown Concentrations of TFM in Water from Four Streams

	Background Color			Sample Values			
	Klett Scale Value			Klett Scale Value			
Stream	Acid	Basic	B/A ratio	Basic (calcd. blank)	Sample	TFM, P.P.M. Calcd.	TFM, P.P.M. Actual
Carp Creek							
Undiluted	..	..	...	97	95	2.2	2.3
Diluted 1:1	35	49	1.40	51	204	5.4	5.5
Diluted 1:2	..	..	...	38	150	3.5	3.6
Milligan Creek							
Undiluted	..	..	...	142	170	5.2	5.3
Diluted 1:1	55	74	1.34	75	173	4.8	4.7
Diluted 1:2	..	..	...	50	115	2.7	2.6
Stokely River							
Undiluted	..	..	...	76	156	4.0	4.1
Diluted 1:1	29	39	1.34	39	110	2.5	2.5
Diluted 1:2	..	..	...	29	220	6.1	6.2
Kaministikwia River							
Undiluted	..	..	...	85	98	2.3	2.2
Diluted 1:1	31	43	1.38	41	153	3.7	3.8
Diluted 1:2	..	..	...	32	208	5.5	5.7

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RECEIVED for review March 21, 1960. Accepted August 4, 1960. Division of Analytical Chemistry, 137th Meeting, ACS, Cleveland, Ohio, April 1960. Work largely supported by the Great Lakes Fishery Commission.

PRINTED IN U. S. A.

PHOSPHOGLUCOMUTASE, PHOSPHORIBOMUTASE, AND PHOSPHOGLUCOISOMERASE OF LINGCOD MUSCLE[1]

GÉRARD-B. MARTIN[2] AND H. L. A. TARR

Abstract

Aqueous extracts of lingcod muscle were saturated with ammonium sulphate and the resulting protein fraction was dialyzed to yield a crude preparation which exhibited marked PGM,* PRM, and PGI activities. When such preparations were flash heated to 55° C and lyophilized, the remaining soluble-protein fraction could be chromatographed on DEAE cellulose columns using gradient elution with tris buffers to separate the PGI from the PGM and PRM activities which were eluted simultaneously. The properties of the pooled PGI fraction thus obtained were similar to those described in the literature from PGI prepared from widely different sources. Instability of the pooled fraction containing PGM and PRM made it difficult to employ this for studies of these enzyme activities, and therefore the crude ammonium sulphate fraction appropriately diluted was used. In this fraction PGM activity was at least 100 times that of PRM activity, and when in the dephospho form both PGM and PRM were inactive unless either GDP, RDP, or DRDP was added. The action of Mg^{++}, CSH, and 8-hydroxyquinoline on PGM and PRM was studied with results which showed marked differences in response of the two enzymes to these agents.

Introduction

It has been pointed out recently that our knowledge of the glycolytic mechanisms in tissues of fishes and marine invertebrates, in comparison with that about warm-blooded mammals, is indeed fragmentary (Gubmann *et al.* (1)). However, much is being done to remedy this defect. Thus, 10 years ago evidence was presented for the occurrence in fish muscles of the hexose phosphates and higher energy phosphorus compounds which are found in mammalian muscles (Tarr (2)). More recently, Cordier and Cordier (3) and Ono and Nagayama (4) have studied fish muscle phosphorylase, and Shibata (5) aldolase from this source. Crude, or fairly highly purified, glycolytic or pentose cycle enzymes recently prepared from fish muscles include PGM (6), PRM (7), phosphoriboisomerase, and ribulose 5-phosphate 3′-epimerase (8). Recent investigations at this Station by Dr. R. A. MacLeod and his collaborators (unpublished) have shown that homogenates of all fish tissues examined, including skeletal muscle, liver, heart, and kidney, contain hexokinase, phos-

[1]Manuscript received August 29, 1960.
Contribution from the Fisheries Research Board of Canada, Technological Station, Vancouver 8, B.C.

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*The following abbreviations are used: Pi, orthophosphate; PGM, phosphoglucomutase; PRM, phosphoribomutase; PGI, phosphoglucoisomerase; G1P, α-D-glucose 1-phosphate; G6P, α-D-glucose 6-phosphate; R1P, α-D-ribofuranose 1-phosphate; R5P, α-D-ribofuranose 5-phosphate; GDP, α-D-glucose 1,6-diphosphate; RDP, α-D-ribofuranose 1,5-diphosphate; DRDP, deoxyribose 1,5-diphosphate; F6P, fructose 6-phosphate; CSH, cysteine; tris, trishydroxymethylaminomethane; DEAE, diethylaminomethylcellulose.

phoglucoisomerase, phosphofructokinase, aldolase, glyceraldehyde 3′-phosphate dehydrogenase, phosphoglyceromutase, enolase, pyruvic kinase, and lactic dehydrogenase. Studies with carp livers indicate that the hexosemonophosphate shunt system is inoperative in this tissue (Tappel *et al.* (unpublished)), but this does not exclude the possibility that this system may function in other fish tissues. The methods of separation and some of the important properties of lingcod muscle PGM, PRM, and PGI are described in the present communication.

Materials and Methods

Dipotassium G1P was obtained from the Sigma Chemical Co., St. Louis, Mo., or from the Nutritional Biochemicals Corp., Cleveland, Ohio. The Sigma product when tested without GDP and dephosphoenzyme (see Experimental section) was not converted to G6P, while the Nutritional Biochemicals product was rapidly converted to G6P under these conditions. This indicated that the latter product probably contains GDP, which is in accord with McCoy and Najjar's recent finding (9). The Nutritional Biochemicals product was used in all but those experiments where tests were made to determine the comparative effectiveness of GDP, RDP, and DRDP. Dipotassium G6P was purchased from the Sigma Chemical Co. The dicyclohexylammonium R1P and tetracyclohexylammonium DRDP used were prepared previously in this laboratory (7, 10). Tetracyclohexylammonium RDP (11) was a chemically synthesized product kindly donated by Dr. Gordon Tener of the British Columbia Research Council. Dibarium GDP was prepared from brewers' yeast (Carling Breweries, Vancouver, B.C.) by the method of Cardini *et al.* (12). It was chromatographically homogeneous in three solvent systems and the total and labile phosphorus content was 98–99% of the theoretical values. It was converted to the sodium salt before use by treatment of solutions with Dowex 50×8 Na^+ resin.

The following compounds were purchased from the sources indicated: L-cysteine hydrochloride (California Foundation for Biochemical Research, Los Angeles); bovine serum albumin (Nutritional Biochemicals Corp.); Tris (Sigma Chemical Co.); 8-hydroxyquinoline (Eastman Organic Chemicals Division, Rochester, N.Y.); resorcinol (Nutritional Biochemicals, Inc.); maleic anhydride (Fisher Scientific Co., Montreal); fructose (Schwarz Laboratories, Mount Vernon, N.Y.), and DEAE type 20 from the Brown Co., Berlin, New Hampshire.

In order to verify the chromatographic homogeneity of the various sugar phosphate esters used, and that of the products arising from the action of the various enzymes on these, descending chromatography on Whatman No. 1 paper for 16 hours at 20–22° C, using *n*-propanol – 28% ammonium hydroxide – water (6:3:1 v/v) (13) was carried out. The position of the phosphate esters was determined using the Hanes and Isherwood (13) spray reagent, and the observed R_f values were checked with literature values or with reference compounds.

Protein was determined by the quantitative biuret method of Kingsley (14) as modified by Snow (15), or, when the protein concentrations were low, by that of Lowry *et al.* (16).

All enzyme reactions were carried out at 30° C. Unless otherwise stated the following assay conditions were employed: PGM activity was determined in solutions containing G1P, 4×10^{-3} *M*; tris–HCl buffer, 0.02 *M* pH 7.5; Mg^{++} (as $MgCl_2$ or $MgSO_4$), 0.001 *M*; and cysteine, 0.01 *M*. The rate of conversion of G1P to G6P was determined by measuring the amount of acid labile G1P present in an aliquot of the reaction mixture made 0.1 *N* with H_2SO_4 and heated at 100° C for 10 minutes before centrifuging and determining Pi by Gomori's method (17).

Unless otherwise stated, PRM activity was determined in solutions containing R1P (4×10^{-4} *M*), and tris–HCl buffer (0.01 *M*) pH 7.4. The velocity of the reaction was determined by removing appropriate aliquots, adding an equal volume of 0.2 *M* perchloric acid, holding for 45 minutes at 20–23° C (a procedure which completely hydrolyzes R1P to yield Pi) (18), and determining Pi in the clear supernates after centrifugation by the Gomori procedure (17).

PGI activity was determined in solutions containing G6P (2.5×10^{-3} *M*) and tris-maleate buffer (0.025 to 0.035 *M*, pH 8.2). An appropriate aliquot of the reaction mixture was removed, an equal volume of 2 *N* HCl added, and the protein removed by centrifugation. The amount of F6P in the clear supernates was determined by the method of Roe (19) using F6P as standard.

The 8-hydroxyquinoline used was added as an alcoholic solution to give a final concentration of 0.001 *M*. A quantity of ethanol identical with that added with 8-hydroxyquinoline had no effect on the reactions.

Experimental

All fractionation and purification procedures were carried out at 0–3° C. Fractionation of aqueous (pH 6.0) extracts of strictly fresh (rigor or prerigor), or of frozen lingcod muscle with ammonium sulphate (pH 7.0) as in previous work carried out at this Station (8, 10) was the basic general method employed to obtain a crude extract which contained the three enzymes. For most of the experiments with PGM and PRM it was found convenient, because of the comparative instability of these enzymes when partially purified, to use a muscle extract which had been saturated with ammonium sulphate, and the precipitate dialyzed against distilled water until free from ammonium sulphate.

Chromatography using DEAE cellulose provided satisfactory separation of PGM and PRM from PGI. The general technique used was identical with that employed in separation of lingcod muscle phosphoriboisomerase and ribulose 5-phosphate 3′-epimerase using a concave gradient elution with tris buffer (8) except that the DEAE was equilibrated with 0.01 *M* tris–HCl buffer pH 7.0. In a typical experiment a crude lingcod muscle extract which had been saturated with ammonium sulphate, dialyzed, heated to 55° C (see below), lyophilized, and centrifuged (1.0 ml containing 33 mg protein nitrogen) was

applied to a 30×1.8 (diam.) cm DEAE column and eluted with a gradient as used in previous work (8). Fractions (6 ml) were collected at a flow rate of 0.6–1.0 ml/minute. The results (Fig. 1) show that the main "protein peaks"

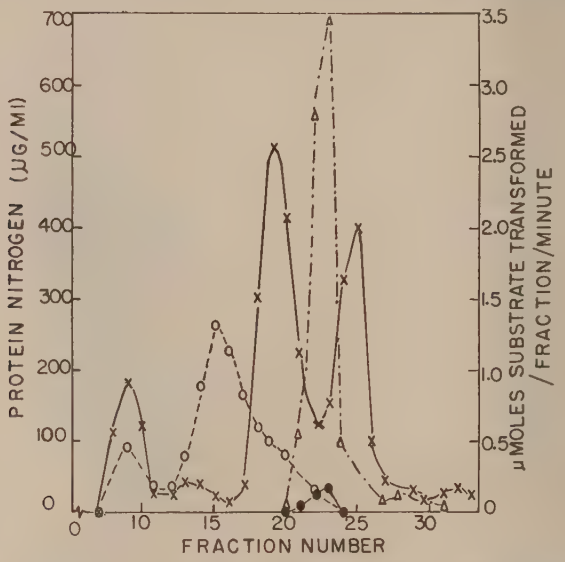

FIG. 1. Separation of PGM, PRM, and PGI using a DEAE column. Protein, ×——×; PGI, ○– – –○; PGM, △–·–·–△; and PRM, ●——●.

did not correspond with the various peaks of enzymic activity. PGI activity was largely distributed between fractions 13–19 inclusive, and PGM and PRM between fractions 21–24 inclusive. The small amount of PGI activity which emerged with the first protein fraction (fractions 7–10 inclusive) could indicate a second distinct PGI enzyme, but, in view of the experience of others with DEAE, would seem to be more probably due to absorption of some of the PGI activity on the protein concentrate emerging in this region. The fractions containing PGM and PRM activity were pooled, but found to be very unstable, losing activity rapidly at 0° C and completely when frozen and thawed. On the other hand the pooled fractions containing PGI retained considerable activity for several days at 0° C and were used in studies of this enzyme.

Partial purification of PGM was also obtained by fractionation with ammonium sulphate at pH 7.0. In a typical experiment 10 ml of a crude water extract of lingcod muscle was fractionated by addition of appropriate amounts of saturated ammonium sulphate solution and the precipitates dissolved in water and dialyzed as usual for testing for PGM activity. The results (Table I) showed that the fractions precipitating between 0.6 and 0.7 saturation with ammonium sulphate had the highest specific activity. A procedure which involved freeze-drying of a dialyzed saturated ammonium sulphate fraction,

TABLE I

Partial purification of PGM from a crude water extract of frozen lingcod muscle using ammonium sulphate precipitation

	Specific activity (units)*	Total protein nitrogen (mg)	Total activity (units)
Crude extract heated at 55° C	5.9	5.4	32.7
0.0–0.5 ammonium sulphate precipitate	0.0	1.6	0.0
0.5–0.6 ammonium sulphate precipitate	1.9	0.7	1.33
0.6–0.7 ammonium sulphate precipitate	17.4	0.58	10.08
0.7–0.8 ammonium sulphate precipitate	6 0	0.48	2.88

*The specific activity is expressed as units per mg of protein N. The unit is defined as 1 μmole of G1P converted to G6P per minute at 30° C under the usual experimental conditions.

re-solution of this in water, centrifugation, flash heating of the supernate to 56° C, followed by rapid chilling and centrifugation to remove coagulated protein resulted in considerable over-all purification (Table II). In view of the instability of PGM and PRM when purified by means of a DEAE column, a

TABLE II

Purification of PGM by heating a crude water extract of lingcod muscle

	Volume (ml)	Total protein nitrogen (mg)	Specific activity (units)*	Total units
Water extract	380	2413	1.33	3,140
Saturated with ammonium sulphate, dialyzed, and centrifuged	250	962	3.63	3,480
Lyophilized, centrifuged	100	465	6.88	3,210
Heated at 56° C	99	262	10.20	2.675

*See Table I.

crude dialyzed saturated ammonium sulphate fraction was used in the following experiments with these enzymes. It was appropriately diluted with 0.1% albumin solution for PGM, and with water for PRM studies.

Relationship between Enzyme Activity and pH

With PGM the normal reaction mixture was prepared without added enzyme, and small portions adjusted to pH values between 5 and 9 by cautious addition of trace amounts of 2 *N* NaOH or HCl. The results show that the enzyme has a fairly sharp optimum about pH 7.5 (Fig. 2).

PRM pH–activity relationships were determined similarly using the reaction mixture previously described. The results (Fig. 2) indicate that this enzyme has a somewhat wider range of activity than PGM.

With PRI the pooled active fractions from a DEAE column were used as source of enzyme. The reaction mixtures were adjusted to different pH values

with either tris–maleate or glycine–NaOH buffers (0.02 *M* final concentration). The results (Fig. 2) show that PGI has a wide range of activity with a broad optimum between pH 8.0 and 10.0.

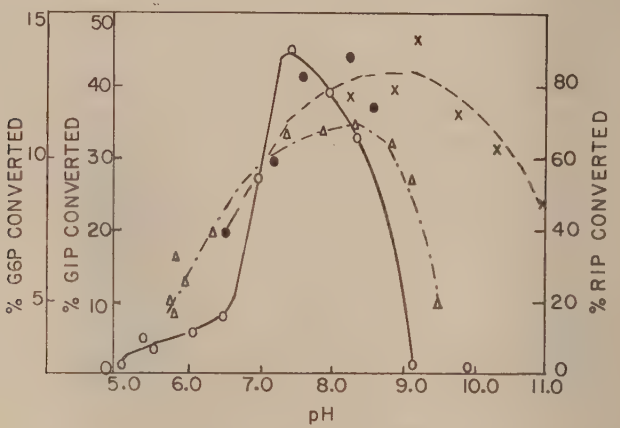

FIG. 2. pH–activity curves. PGM, O——O; PRM, △-·-·-△; and PGI, tris–maleate (●- - ●) and glycine–NaOH (×- - ×) buffers. Conditions: PGM, 1.2 μg protein N in 0.25 ml for each point, reaction time 6 minutes; PRM, 170 mg protein N in 0.375 ml for each point, 40-minute reaction time; PGI, 1.25 μg protein N in 0.10 ml for each point, reaction time 8 minutes.

Equilibrium

The conditions used in determining equilibrium in the PGM system were identical with those employed for the pH–activity data, the reaction being carried out at pH 7.4. The results (Fig. 3) show that the reaction rapidly reaches an equilibrium at which the ratio of G1P:G6P is approximately 5:95 For PRM the results (Fig. 3) show that in this system the equilibrium R1P to R5P was about 15 to 85. With PGI the conditions were identical with those used for determining the pH–activity relationship. The results (Fig. 3) show that at equilibrium the ratio of G6P to F6P was 38 to 62.

Effect of Mg^{++} *and Cysteine*

The effect of Mg^{++} on PGM was determined at pH 7.3. The enzyme employed for this experiment was a freshly prepared eluate from a DEAE column. It was used at a concentration of 175 μg protein N/ml, which was considerably higher than that required for the more stable crude preparation employed in most experiments. The results (Fig. 4) indicate a marked requirement for Mg^{++}, optimum activation occurring at 1×10^{-3} *M* concentration. The effect of cysteine was determined under similar conditions, the Mg^{++} concentration being 1×10^{-3} *M*. PGM also exhibits a strong requirement for cysteine with optimum activation at about 1×10^{-2} *M* (Fig. 5).

For PRM the effect of Mg^{++} and cysteine on activity was determined under

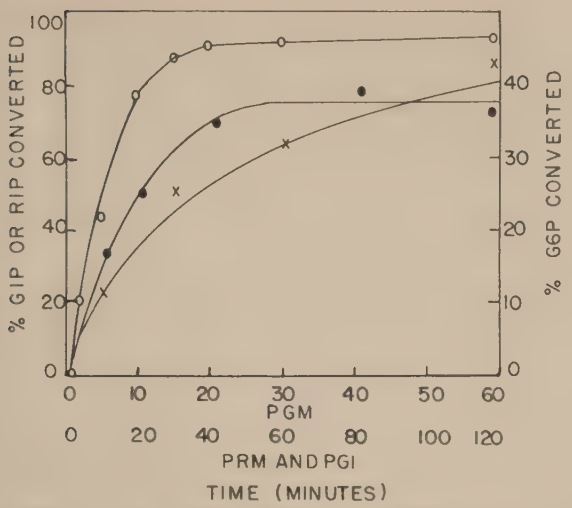

FIG. 3. Equilibrium between G1P and G6P in the PGM system, ○——○; between R1P and R5P in the PRM system, ×——×; and between G6P and F6P in the PGI system, ●——●. Conditions: PGM, pH 7.4, 4.7 μg protein N per ml; PRM, pH 7.3, 440 μg protein N per ml; PGI, pH 8.2, 125 μg protein N per ml.

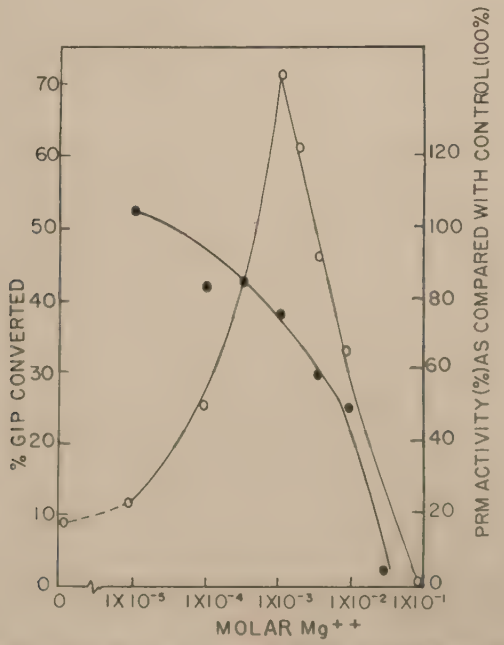

FIG. 4. Effect of Mg^{++} on the activity of PGM, ○——○, and PRM, ●——●. Conditions: as for Fig. 3.

conditions identical with those described for the previous experiments with this enzyme. The results (Figs. 4 and 5) show that PRM differs strongly from PGM in that neither Mg^{++} nor cysteine activates the enzyme. Both cause inhibition in the higher concentrations used.

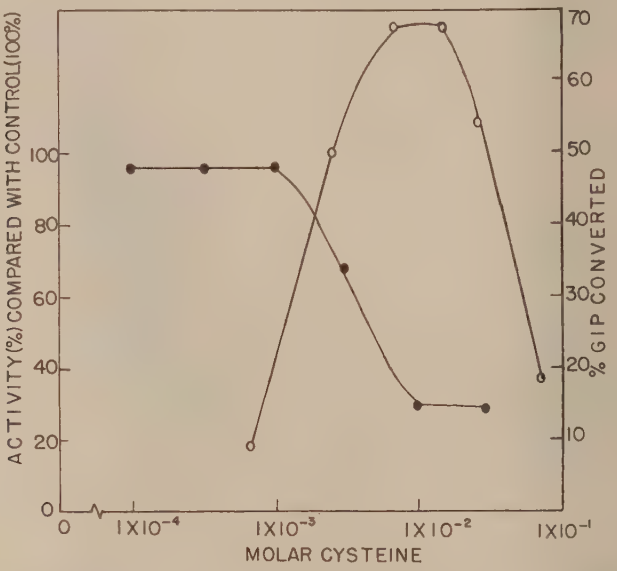

FIG. 5. Effect of cysteine on the activity of PGM, O——O, and PRM, ●——●. Conditions: as for Fig. 3.

Effect of GDP, RDP, and DRDP on PGM and PRM Activity

Crude enzyme (5 ml) to which 10 mg of G1P (Sigma) was added (20) was dialyzed 1 day at 0° C against several changes of a solution containing 0.01 *M* CSH and 0.001 *M* Mg^{++}, and the procedure then repeated. The enzyme was dialyzed a third day without addition of G1P and was then presumably entirely in the dephospho form. It was used in the following experiments designed to determine the effect of the three diphosphate esters on activity of PGM and PRM.

It was found (Fig. 6) that PGM was inactive in the absence of the diphosphate esters, and that all three exerted optimum activation at a concentration of 1×10^{-5} *M*, under the experimental conditions. A marked difference in activity was noted, namely GDP>RDP>DRDP. A similar experiment carried out with crude enzyme which had been dialyzed in the usual manner without addition of G1P (11 μg protein N per test) indicated that it was also largely in the dephospho form since it was almost inactive without added GDP. In this experiment the optimum concentration of GDP was also 1×10^{-5} *M*. Other studies indicated that the optimum GDP concentration is dependent on

the substrate (G1P) concentration and this finding is in accord with the results of Najjar and his collaborators (21, 22).

The influence of the three diphosphate esters on PRM activity is shown in Fig. 7. Without added diphosphates no conversion of R1P to R5P was observed. The results indicated that the activating effect of the diphosphates was

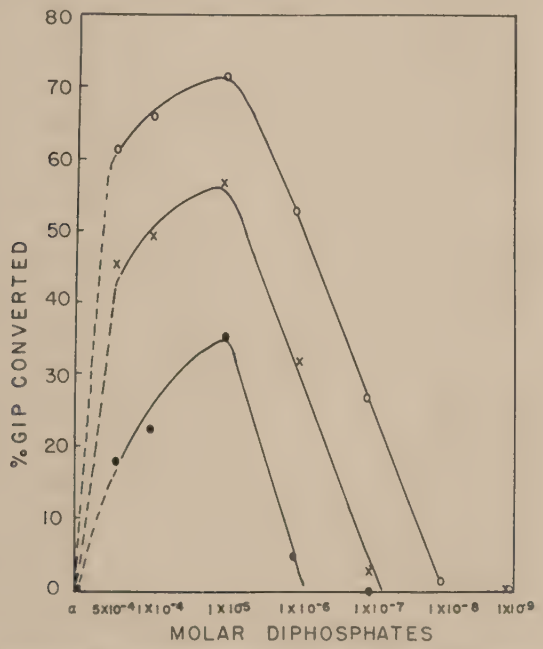

FIG. 6. Effect of GDP, RDP, and DRDP on PGM activity. Conditions: 44 μg protein N. G1P (Sigma) 1 μmole, and the specified concentrations of diphosphate esters in 0.2 ml reaction mixture for each point; reaction time 4 minutes.

as follows: RDP > DRDP > GDP, but the differences were comparatively small. However, in the presence of 8-hydroxyquinoline the differences in effectiveness of the diphosphate esters was marked. Thus one experiment (900 μg protein N ml under standard conditions) showed that, when used in 1×10^{-5} *M* concentration and in the presence of 8-hydroxyquinoline, the effectiveness of the diphosphate esters in stimulating PRM activity was greatly enhanced, but the comparative effectiveness of the three compounds was in the same order as that found without 8-hydroxyquinoline.

With PGM the effect of 8-hydroxyquinoline was exactly the reverse of that found with PRM. Thus, with dephosphoenzyme under the usual conditions (44 μg protein N ml^{-1} and in the presence of an optimum concentration of GDP (1×10^{-5} *M*) 8-hydroxyquinoline caused about 20% increase in activity.

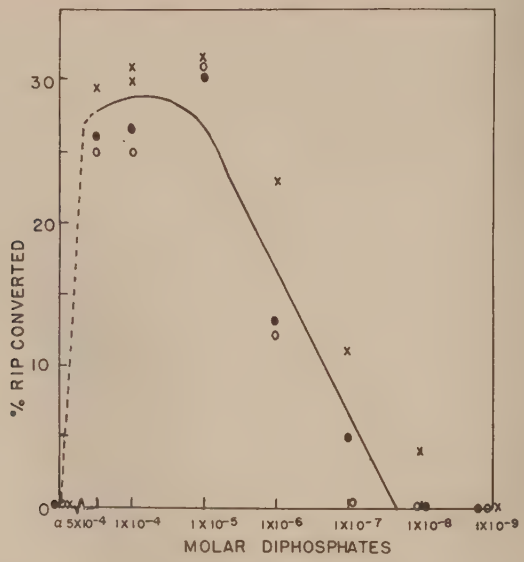

FIG. 7. Effect of GDP (○——○); RDP (×——×); and DRDP (●——●) in PRM activity. Conditions: enzyme 110 μg protein N, R1P 0.5 μmole, and the specified concentrations of the diphosphates in a final 0.2-ml volume for each point. Reaction time 10 minutes.

Discussion

The properties of PGM as observed in lingcod muscle are very similar to those found for PGM from mammalian muscle. The equilibrium of 95% in favor of G6P is in close agreement with values obtained elsewhere (9, 22, 23, 24) with other tissues. The results obtained for Mg^{++} requirement are in agreement with findings of previous workers. Mg^{++} was found to be essential for, or at least a very strong activator of, PGM by a number of investigators (25, 26, 27, 28). The present findings correspond almost exactly to the results of Najjar (22) with crystalline PGM from rabbit muscle. The CSH requirement of rabbit muscle PGM was studied by Najjar (22) with results similar to those found in the present work. A requirement for CSH was also demonstrated by Sutherland (29). However, Ramasarma *et al.* (23) could not find any activation of this enzyme by CSH when working with a partially purified enzyme from green gram. The optimum pH of approximately 7.5 found in the present work is in good agreement with the results of different workers with rabbit muscle PGM (22, 26, 30).

The absolute requirement of PGM for GDP, first demonstrated by Najjar and Pullman (31), has been verified for the fish muscle enzyme. It has, moreover, been shown that, in order of decreasing activity, RDP and DRDP also catalyze the reaction. However, this is not unexpected since it is known that rabbit muscle PGM will cause a reversible reaction between R1P and G6P in

which both GDP and RDP are in equilibrium (32) and will also effect a similar reaction between mannose 1-phosphate and GDP (33). Optimum activation of PGM was occasioned by 1×10^{-5} *M* concentrations of the three diphosphate esters. Other experiments have shown that this optimum varies with the concentration of substrate (G1P), which is in accord with previous studies on this enzyme (9, 20).

The properties of lingcod muscle PRM are also very similar to those of the corresponding enzyme in other tissues. The equilibrium of about 85% in favor of R5P formation is in good accord with Klenow's (34) findings. The range of pH for optimum activity was observed to be between about 7.0 and 8.5. This compares with the optimum pH 7.5 found by Klenow (34) but differs from the results of Guarino and Sable (35), who found a pH optimum of 6.7 to 7.2 for the bovine uterus enzyme. The present results showed no significant activation with Mg^{++} and a very strong inhibition with high concentrations of this ion. Similar findings were obtained by Guarino and Sable (28, 35), although Klenow (34) found an activation by Mg^{++}. The inhibitory effect of high $MgSO_4$ concentrations is explained by the competition of the anions with R1P (or G1P) for the enzyme (36). Cysteine behaved similarly to Mg^{++} and this compares well with the findings of Guarino and Sable (35) with PRM bovine uterus, though the same authors found an activation by cysteine when working with human blood cells (28). These inconsistencies in activity of CSH may be due to the metal binding activity of the protein itself in experiments with PRM where the protein concentration was comparatively high.

PRM was inactive in absence of the diphosphate esters. The very marked differences in activating effect of these esters observed with PGM were not observed with PRM, but in general their effectiveness was as follows: RDP> DRDP>GDP. This difference was accentuated by the presence of 8-hydroxyquinoline, the inhibiting effect of which was markedly overcome by the diphosphates. Thus, under the usual experimental conditions, the three esters assumed the same order of effectiveness as noted above, but the differences were much more marked. As noted by Klenow (34) there is as yet no explanation for the effect of 8-hydroxyquinoline.

According to the present findings PGM and PRM have points of resemblance with the corresponding mammalian enzymes. Fish muscle PGM exhibits about the same properties with regard to equilibrium, optimum pH, and requirement for Mg^{++} and cysteine. The properties of PRM in mammalian smooth muscle (29) are not well established and it is therefore impossible to compare fish muscle PRM with the corresponding mammalian enzyme.

So far as can be ascertained, Guarino and Sable (35) are the only investigators who have presented data which, with bovine uterus preparations at least, they feel may indicate that PGM and PRM are different enzymes. The present work has shown significant differences in the effect of 8-hydroxyquinoline, Mg^{++}, and cysteine on PGM and PRM activities present in a crude lingcod muscle enzyme preparation. Though, on a protein basis, PGM activity

was usually at least 100 times that of PRM activity, the instability of purified preparations made it difficult in the present work to compare specific activities of PRM and PGM during purification procedures. Until a highly purified PGM preparation devoid of PRM activity, or vice versa, has been obtained, it would seem unwise, on the basis of the present limited evidence, to suggest that PGM and PRM are distinct enzymes.

At equilibrium, a ratio of 38% F6P to 62% G6P was found with PGI. This is similar to the following ratios reported by other investigators with PGI from widely different sources, namely: 32:68 (35); 40:60 (36); 29:71 (37). The pH range for optimum activity of the enzyme was also similar to that reported by the above investigators.

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[Reprinted from THE JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, February, 1961 Issue, Vol. XXXVIII, No. 2, pp. 80–84]

The Effect of Storage Temperature and Antioxidant Treatment on the Chemical and Nutritive Characteristics of Herring Meal

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The effects of storage temperature and antioxidant treatment on the chemical and nutritive properties of herring meal were studied. The following observations were made:

1. Low-temperature (−20°C.) storage promoted more rapid decrease in ether extractability and in iodine value of the ether extract than did storage at 25.5°C.
2. Antioxidant treatment prevented the decrease both in ether extractability and iodine value of the extract.
3. Binding of the lipid into a complex from which the lipid was extractable with acetone only after HCl treatment occurred early in the storage period. After six weeks the amount of lipid in this fraction decreased, presumably as a result of further oxidation and polymerization into more refractory compounds.
4. Pepsin digestibility tests showed that BHT-treated meal contained the lowest amounts of undigestible nitrogen. Meals subjected to −20°C. contained the most undigestible nitrogen.
5. All meals were similar in nutritive value as protein or vitamin-B supplements in chick diets, composed of natural ingredients, even after storage for nine months. As the sole source of protein in a purified diet, the meal kept at −20°C. from time of processing promoted slower growth than the other meals.

HERRING MEAL used in livestock and poultry rations contains a variety of lipids. Commercially-produced herring oil is a relatively unsaturated fat, and the lipids remaining in the press-cake are even more unsaturated (1). Because of the reactivity of the unsaturated fatty components in the press-cake, the chemical and nutritive characteristics of press-cake might be expected to undergo considerable change in the course of processing and subsequent storage of the meal.

Stansby (2) observed that the apparent fat content of fish meal, as determined by ether extraction, decreases even during a short storage period. Almquist (3) reported that in sardine meal the extractability of fat and digestibility of protein decreased during storage and concluded that the changes noted were due to oxidation since they were not observed in samples which were stored in sealed glass ampoules. Meade and McIntyre (4) and Aure (1) reported that the addition of butylated hydroxytoluene (BHT) to menhaden and herring meal, respectively, retarded oxidative changes in the meal.

The following experiments were designed to determine and follow the changes which occur in herring meal stored under different conditions and to ascertain what effect antioxidant treatment might have upon any changes that normally take place in meals under ordinary conditions of storage.

Procedure

The herring meals for the experiment were prepared on December 9, 1958, at a commercial reduction plant. The meal required for the experiment was taken directly from the bagging operation, and portions were treated as follows:

- A Stored at 25.5°C. This meal will be referred to henceforth as the "normal" meal.
- B Stored at −20°C.
- C Spread out on trays to facilitate cooling in a freezer room at −20°C. over-night and then bagged. Stored at 25.5°C.
- D Spread out in a freezer room at −20°C. over-night and then bagged. Stored at −20°C.
- E Stored at 25.5°C. for one week. BHT[1] was then added at a level of 0.15%. Stored at 25.5°C.
- F BHT added immediately to the hot meal at 0.15%. Stored at 25.5°C.

The meals were all stored in polyethylene-lined multiwall paper bags.

Samples of meals A and D were taken for immediate chemical analysis and samples of the remaining meals were withdrawn for analysis 10 days after preparation. All meals were sampled on January 16, March 16, and September 24, 1959, for chemical analysis and biological testing with chicks.

Chemical Tests. Weighed samples of the meals were dried *in vacuo* at 99–100°C. for 5 hrs. They were then extracted with peroxide-free diethyl ether in a Soxhlet apparatus for 20 hrs. Iodine numbers (Hanus) were determined on the ether extracts. The ether-extracted samples were then extracted with acetone. The acetone-extracted samples were next digested with HCl and extracted again with acetone according to the AOAC procedure (5). The two successive acetone extracts were weighed separately.

Samples of the whole meals and of ether-extracted meals were subjected to pepsin hydrolysis (6) in order to determine the digestibility of the protein content of the meals.

Biological Tests. The comparative protein values of the meals were determined in growth studies with chicks. The meals were used to supplement the cereal protein in a basal diet composed of natural ingredients and also as the sole sources of protein in a purified diet. The total protein content of both the natural and the purified diets was 20%. In the final test conducted with meals that had been in storage for nine months, an additional and more critical assay of the relative protein supplementary value of the meals was carried out. In this final test the meals were fed in natural diets containing only 14% total protein.

[1] A finely divided product: 90% <100 mesh, 70% <300 mesh.

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TABLE I

Composition of Diets

Purified diet	20% Protein /100 lb.		
Herring meal	27.33	lb.	
Cornstarch	64.02	lb.	
Hydrogenated vegetable oil	4.5	lb.	
Choline chloride (25%)	0.5	lb.	
Ground cellulose	2.0	lb.	
Potassium phosphate, dibasic	0.4	lb.	
Iodinated salt	0.5	lb.	
Limestone	0.75	lb.	
Manganese sulphate	6.0	g.	
Inositol	45.4	g.	
Biotin	0.0091	g.	
Menadione	0.0454	g.	
Pyridoxine HCl	0.182	g	
Folacin	0.182	g.	
Riboflavin	0.361	g.	
Calcium pantothenate	0.908	g.	
Thiamin HCl	0.454	g.	
Niacin	4.54	g.	
P-aminobenzoic acid	9.08	g.	
α-Tocopheryl acetate	0.68	g	
Ascorbic acid	10.0	g.	
Vitamin D_3	120	I.C.U./lb.	
Vitamin A	2000	I.U./lb.	
Natural diet	**20% Protein /100 lb.**		**14% Protein /100 lb.**
Herring meal	16.0	lb.	5.0 lb.
Ground yellow corn	82.0	lb.	92.5 lb.
Bonemeal	0.5	lb.	1.0 lb.
Limestone	1.0	lb.	1.0 lb.
Iodized salt	0.5	lb.	0.5 lb.
Manganese sulphate	0.02	lb.	0.02 lb.
Menadione	0.022	g.	0.022 g.
Niacin	0.6	g.	0.6 g.
Calcium pantothenate	0.21	g.	0.21 g.
Riboflavin	0.065	g.	0.065 g.
Vitamin D_3	120	I.C.U./lb.	120 I.C.U./lb.
Vitamin A	2000	I.U./lb.	2000 I.U./lb.
	[a]		[a]

[a] The following vitamins were added in tests of the meals as protein supplements: riboflavin 1.95, calcium pantothenate 6.3, niacin 18.0, pyridoxine HCl 1.95, biotin 0.06, folacin 0.375, vitamin B_{12} 0.006, choline chloride 900, α-tocopheryl acetate 6, and thiamin HCl 1.2 mg. lb.

The vitamin B-complex content of the meals was compared when the meals were incorporated in a basal diet marginal in the vitamin B-complex. The formulae of the basal diets are given in Table I.

The chicks used in all experiments were 10–14 days old and were standardized as to weight. They were New Hampshires of the same strain in all the experiments. The parent stock was maintained on a ration which was marginal in vitamin content in order that the carry-over to the chicks should be as constant as possible and not so high as to preclude the possibility of response to vitamin supplements. Prior to standardization and the feeding of the experimental diets the chicks were given a chick starting-diet which permitted a moderate rate of growth.

The chicks were reared in battery brooders and were given the experimental diets and water *ad libitum*.

Chemical Tests. The normal meal A contained 73.2% crude protein, 10.7% ash, 9.5% ether-extractable fat, and 7.0% moisture when analyzed on the day after manufacture. All the meals were analyzed for moisture content at the time of each set of analyses. The results of the various analyses expressed on the basis of the moisture-free meals are shown in Table II and Figures 1 and 2. All the values given below are likewise in terms of the moisture-free meals.

The percentage of ether-extractable material in the dried fish meal was initially 10.2. In the case of the two meals treated with BHT (meals E and F) there was no decrease in the amount of ether extract even after storage at 25.5°C. for nine months. Meal D, which was spread out to cool at −20°C. over-night, then rebagged and held at −20°C. showed the most rapid drop in ether-extractable material. Meal B, which was stored at −20°C. but not spread out for rapid chilling, showed a similar content of ether-extractable material (8.7%) at the end of nine months of storage at −20°C. In the case of the latter meal however the decrease in ether-extract was gradual rather than rapid during the first six weeks after processing as in the case of meal D. Meal C was chilled after processing but was stored at 25.5°C. after being rebagged. The decrease in ether-soluble material in the first six weeks for this meal was intermediate between the decrease occurring in meals B and D. From the sixth to the fourteenth week of storage the percentage of ether-extractable material decreased at a faster rate in meal B (−20°C.) than in meal C (25.5°C.).

The iodine values (Figure 2) of the ether extracts showed a general relationship to the amount of ether-extractable material. The iodine value of the ether extracts from the freshly-processed herring meal was 134–135. BHT not only prevented a decrease in the ether-solubility of the fat in the original meal, but also prevented to a considerable degree the drop in the iodine value of the ether-extractable material. After nine months of storage there was no difference between the iodine values (126 and 128, respectively) of the ether extracts of the meal treated immediately after processing with BHT and that of the meal treated one week after processing. There was however a difference between the two meals in the rate at which the iodine value decreased during storage. In the meal kept without BHT the I.V. decreased more rapidly during the first week, but thereafter there was little change. The meal which was treated while still hot from the drier showed a higher unsaturation (I.V. 143) in the ether extract than did the normal meal

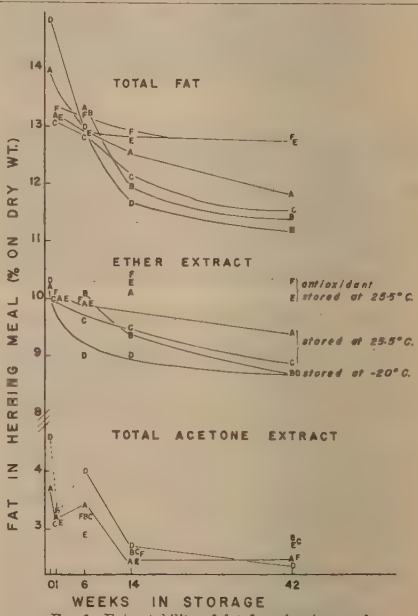

FIG. 1. Extractability of fat from herring meal.

TABLE II

Pepsin Digestibilities and Relative Biological Protein Values of Stored Herring Meals

Pepsin Digestibility	Date of sampling	Normal stored 25.5°C.	Normal stored −20°C.	Chilled stored 25.5°C.	Chilled stored −20°C.	BHT after 1 week stored 25.5°C.	BHT to hot meal stored 25.5°C.
% Indigestible protein (on dry-weight basis) determined by pepsin digestion of whole meal	Dec. 10	4.8	....	....	4.7	....	....
	Dec. 19	5.1	....	5.3	....	5.1	4.5
	Jan. 16	5.4	6.2	5.8	6.4	4.9	4.6
	Mar. 16	5.0	6.1	5.9	6.4	5.1	5.6
	Sept. 24	5.8	6.2	6.3	6.4	5.0	4.7
% Indigestible protein (on dry-weight basis) determined on ether-extracted meal.	Dec. 10	3.9	....	....	3.7	....	....
	Dec. 19	4.6	....	4.7	....	4.7	4.3
	Jan. 16	4.4	4.6	4.7	5.0	4.1	3.9
	Mar. 16	4.7	6.0	4.9	6.3	4.6	4.4
	Sept. 24	5.2	5.5	5.5	5.3	4.7	4.5

Ranking of meals according to growth rates of chicks fed the meals as the sole source of protein in a synthetic diet.[a]

Dec. 19	C^{103}	E^{102}	A^{100}	B^{98}	F^{92}	D^{80}
Jan. 16	F^{101}	B^{100}	A^{100}	C^{99}	E^{97}	D^{85}
Mar. 16	F^{106}	D^{105}	B^{102}	E^{101}	A^{100}	C^{100}
Sept. 24	A^{100}	E^{95}	F^{95}	D^{93}	C^{93}	B^{90}

[a] Herring meals not underscored by the same line are significantly different (P 0.05).

on the day after manufacture. The iodine values for the two meals became similar sometime after three months at 25.5°C. The normal meal A and meal C, which was cooled rapidly to −20°C. immediately after processing and was subsequently stored at 25.5°C., showed similar rates of decrease in the iodine value of the ether extracts after the first week. The iodine value of the rapidly chilled meal D, which was stored at −20°C., however fell sharply to 79 during the first month of storage as compared to meal B, which was stored at −20°C. but not chilled quickly to that temperature. By the end of the storage period the iodine values of the extracts from meals B and D were very similar (66 and 62, respectively).

The drop in the total fat content of the meals (Figure 1) from 14.8 to 13.0% in the first month after processing was due as much to a decrease in the amount of the first acetone extract as to a decline in the ether solubility of the fat in the meal. Acetone extraction of the normal meal and the meal chilled over-night, which had been extracted with ether on the day after the meals were prepared, showed higher values (1.49 and 2.30%, respectively) than any of the meals sampled after one week. After three and nine months of storage the acetone extract was considerably lower, and there was not a great deal of difference among the percentages found in the various samples. The material soluble in acetone only after acid treatment varied comparatively little during the first week after preparation of the meals. During the first six weeks this fraction of acetone-soluble material decreased in the two meals to which BHT had been added. Thereafter the amount of this material decreased in all of these meals. From three to nine months the percentage of the HCl-acetone-soluble fraction diminished rapidly so that the average of all the meals had dropped from an original value of 2.26 to 1.77% at the time of the final analysis. The variation in the total acetone (acetone plus HCl-acetone) extract over the nine-month storage period is shown in Figure 1. The amount of lipid in the meals that was not ether-soluble but which was extractable with acetone, either before or after treatment with HCl, showed marked changes up to three months but relatively little change during the next six months of storage.

On the basis of pepsin hydrolysis of ether-extracted samples the original digestibility of the protein of the meals was 96%. After storage this dropped to 94–95% (Table II). Digestibility of protein of the meals as judged by the results of *in vitro* pepsin hydrolysis demonstrated that antioxidant treatment somewhat inhibited the formation of indigestible nitrogenous compounds. Immediate application of BHT gave consistently better results than application of the antioxidant one week after processing. This was true whether pepsin digestibility of the protein was determined on the whole meal or on ether-extracted samples. Meals B and D, which were kept at −20°C., showed the most rapid formation of undigestible nitrogenous compounds. Meals A and C were intermediate in the drop in digestibility of the protein.

Biological Tests. When the meals were tested in diets composed of natural ingredients, there were no statistically significant differences in the responses obtained to the various meals. This was true whether the meals were considered as a source of supplementary protein only or were considered as a vitamin B-complex supplement as well. In the final test on the meals after storage for nine months there were no differences in the protein supplementary value of the meals, either as supplements to a practical type of diet containing a total of 20% protein or when used to supply only 3% of protein to give a total protein level of 14%.

In contrast to the results of the chick assays for supplementary protein quality, there were differences in the response obtained with the different meals when they were fed as the sole source of protein in a purified diet. The chicks were not standardized at the same weight in all tests, and the duration of the experiments varied somewhat. It is therefore not possible to make comparisons among the absolute weights of the chicks in experiments conducted on different dates or among different experiments on a given date. Within each experiment the final weights of the chicks were subjected to statistical analysis and significance was tested according to Duncan's multiple-range test (7). Table II gives the average weights of the chicks within each test, relative to the average weights of the chicks receiving the normal meal which was assigned, for purposes of comparison, a value of 100. In none of the tests conducted over the storage period was any of the meals statistically superior to the normal meal A. Except in the test on the meals after storage for three months when there were no significant differences in the responses to the meals, the chicks fed meal D (the chilled and frozen meal)

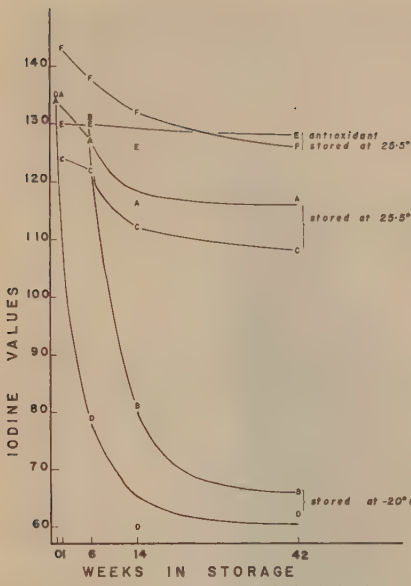

FIG. 2. Iodine values of ether-extractable fat in herring meal.

were significantly lighter than the chicks fed the normally-handled meal.

Discussion

The most striking alterations in the herring meals were those occurring in the meals subjected to a temperature of −20°C. An initial low temperature appeared to favor the development, during the first month of storage, of compounds which were no longer split by HCl or of compounds which did not yield acetone-soluble products after HCl treatment. The meals treated with BHT showed no increase in the amount of material which was acetone-extractable only after HCl digestion. The effect would seem to have been against formation of co-polymerization products, from which the lipid fraction could be extracted with acetone after splitting with HCl rather than through the blocking of further change in such products. The effects noted in the meals, which were spread out to promote rapid chilling after processing, were not due merely to the exposure to air during the procedure. Comparison of the progressive decrease in iodine values of the ether-extracts of the different meals shows a more rapid decline in meal D, which was chilled and then stored at −20°C., than in any of the other meals. In meal B, which was not chilled but which was stored at −20°C., the I.V. had not decreased appreciably after six weeks of storage but thereafter dropped rapidly until, after nine months, it was similar to that of the meal which had been chilled before being put into low temperature storage. Furthermore the meal which was chilled and then kept at 25.5°C. showed an initial drop in iodine value, but after one week the rate of decline was similar to that occurring in the normal meal. It may accordingly be concluded that the lower temperature favored rapid oxidation of the unsaturated fatty components of the meal. In this connection Astrup (8), using oxygen absorption as a measure of oxidation in herring meals, noted that the progress of oxidation differs depending on the temperature. Astrup suggests that the minimum range which he observed indicates the formation of antioxidants in the meal with elevated temperature. Lea, Parr, and Carpenter (9) likewise reported a decrease in the rate of oxidation in herring meal with higher storage temperatures and suggested an accumulation of oxidation inhibitors. They consider it possible that "whereas oxidation of the lipid probably proceeds further at lower temperatures, coupling of the oxidized oil to the protein (or its destruction) might be greater at higher temperature."

Whatever the nature of the reactions favored at the different temperatures it is clear that so-called accelerated tests, employing higher temperatures than those normally encountered in the storage of herring meal, are not applicable for a study of the storage stability of fish meal. Moreover the rapidity with which oxidative changes occurred when the meals were subjected to a temperature of −20°C. renders it difficult to establish a control for the study of changes taking place during the storage of fish meal.

In an earlier study (10) the biological value of experimentally-prepared herring meal, dried at low temperature (37–43°C.), was compared with that of a normal commercial meal and a commercial meal that was overheated. The meals were stored at −25°C., 21°C., and 37°C. for a year and tested for their nutritive value as protein supplements in chick diets at the end of that time. Samples of the meals were extracted with hexane prior to storage to determine what changes in nutritive value may occur in fat-free meals during storage. By comparison of the nutritive value after storage of the meals of normal fat content with that of the extracted meals the role of fat in any changes in nutritive value could be assessed. The only conclusion drawn at that time from the results obtained was that storage at a temperature as high as 37°C. had no effect on the nutritive value of herring meals as protein supplements. Reconsideration of the data in view of the results of the present investigation enables some additional observations to be made. After storage at 21°C. the extracted low-temperature meal did not support as rapid growth as did the extracted meals dried at higher temperature. When the meals were stored at −25°C., the extracted low-temperature meals again did not promote as rapid growth as did the extracted meals that had been dried at higher temperatures. Of the unextracted meals the overheated commercial meal gave the fastest rate of growth. It was also observed (11) that controlled heating of herring meals dried at a low temperature did not lower the nutritive value of the meals and that in many instances chicks grew better with the heated meals. From the results of these various experiments it appears that certain changes in herring meal occur at a faster rate or take a different course when the processing and storage temperatures are low. The absence, in the present experiment, of significant differences in the biological responses obtained to the meals subjected to different temperatures is probably accounted for by the fact that, in contrast to the pre-

vious experiments, none of the meals was processed at low temperature.

Antioxidant treatment of the meal prevented a decrease in ether-extractability of the fat and to a large extent prevented a decrease in iodine value of the ether-extractable fat. The increase noted with some of the meals in the HCl-acetone extract during the first six weeks of storage appears to represent the formation of lipid complexes with protein which are split by HCl treatment to yield an ether-soluble lipid component. BHT apparently prevented the formation of these complexes. By inhibiting oxidation of the unsaturated lipid in the meal, BHT may have retarded the various types of polymerization that have been postulated for systems containing fat and protein and subjected to oxidative conditions (12,13, 14). It may be noted that postponing the BHT treatment of the meals until one week after manufacture permitted oxidative reactions in the fat that were, in this short time, apparently accompanied by polymerization with protein. Consequently the *in vitro* digestibility of the meal treated immediately with BHT was better throughout the entire storage period than that of the meal treated one week after manufacture. It is interesting that the advantage obtained with BHT treatment was constant at each sampling date. Although the amount of ether-extractable lipid remained similar for the two antioxidant-treated meals during storage, the iodine value of the meal stored without BHT for the first week dropped in the course of that week but did not change appreciably thereafter. Immediate addition of BHT to the hot meal, on the other hand, inhibited oxidation so that after one week the iodine value was higher than that of the normal meal on the day after manufacture.

Insofar as the biological tests are concerned, it should be noted that the diets fed were formulated so that in no instance was the available energy content of the fish meal a factor in the response of the chicks. The extent to which the fat content of the meals was utilized by the chick did not therefore affect the response of the chicks. With the purified diets however, in which a relatively high level of fish meal was fed, it is possible that the depression in growth noted with meal D could have resulted from destruction of vitamins in the diet by the oxidizing fat in the meal. Although all of the vitamins were present in considerable excess, they are especially labile in this type of diet. A separate study is being made of the nutritive value of the fat content of fish meal prepared and stored under different experimental conditions.

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[Received April 14, 1960]

PRINTED IN THE U.S.A.

ON STRUCTURE, ENTRAINMENT, AND TRANSPORT IN ESTUARINE EMBAYMENTS

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ABSTRACT

When fresh water enters the surface of an embayment it moves persistently seaward. Enroute it entrains seawater from below to form a halocline, in which the salinity increases with depth and to seaward. Wind mixing creates a nearly homogeneous zone in the upper part of the halocline. Below the halocline there is a nearly homogeneous lower zone in which sea water intrudes at a rate sufficient to supply the demand for entrainment. This fresh water transport system is superimposed on the tides which ebb and flood at all depths. There is a surface of no net motion in the halocline above which the net transport is seaward and below which it is inwards.

Entrainment is the preferential upward transfer of water from the lower to upper zone. The downward mixing of fresh water is defined by the lower limit of the halocline, which is a surface of unidirectional upward transfer.

The zone structure is accurately defined by plotting salinity as a function of the logarithm of depth.

The seaward volume transport through the upper zone and halocline, the inward transport through the lower zone, and the upward transfer through the interzone boundary may be computed in terms of the continuity of fresh water, where the rate of input is known. Where it is not known, the equations are solved by introducing the difference of dynamic height across the embayment. The features of structure and transport may be defined by application of normal oceanographic data.

This paper is presented in recognition of the inspiration and leadership of Thomas G. Thompson.

THE ESTUARY MODEL

In an estuarine embayment the input of fresh water exceeds the losses by evaporation and/or freezing.

From his study of Alberni Inlet (Fig. 1), Tully (1949) concluded that fresh water entering the surface of such an embayment moves persistently seaward. Enroute it entrains sea water from below to

REPRINT FROM JOURNAL OF MARINE RESEARCH, VOLUME 17, 1958

FRB No. 642

form a halocline in which the salinity increases with depth and to seaward. Usually the upper part of the halocline, mixed to or nearly to homogeneity by wind, is called the upper zone. Below the halocline there is a nearly homogeneous lower zone in which sea water

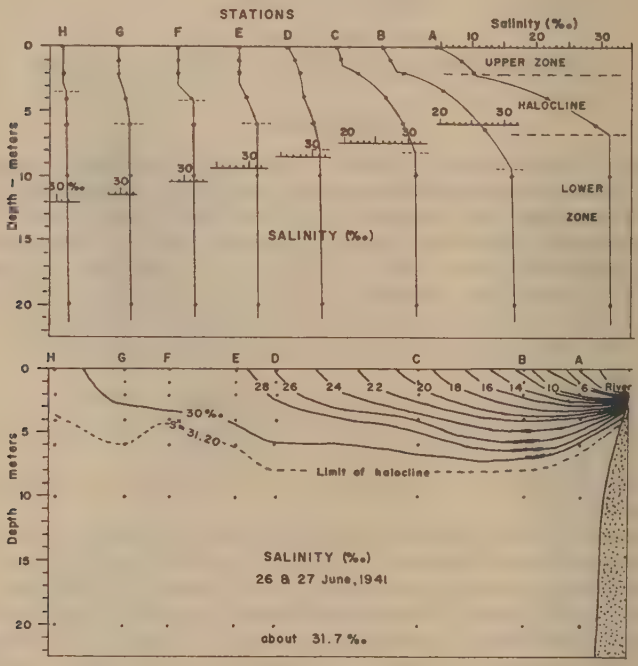

Figure 1. Salinity structure in Alberni Inlet, B.C.

intrudes at a rate sufficient to supply the demands for entrainment. In the steady state (*i. e.*, through a time interval longer than a tidal day) the fresh water is displaced (flushed, transported) seaward at the same rate as it enters the embayment. Seawater is continuously transferred upward through the halocline along the path of transport. It is mixed with the fresh water and conserved in the upper zone and in the halocline, and it moves seaward at the same rate as the fresh water.

It is of some interest to re-examine the features of this model in the light of more recent knowledge.

Fresh Water Transport Mechanism. Such a transport mechanism can only recognize the difference in density between the zones. It requires a continuous source of light water (fresh water from precipitation and land drainage), a continuous source of dense water (the ocean), and a sink for the mixed light and dense water (the ocean).

The greatest concentration of light (fresh) water is at the head of the system. Hence there is a gradient of increasing mean density seaward. This is compensated by a seaward slope of the sea surface which is the seaward driving force in the upper zone. The driving force in the lower zone is provided by an opposite slope of the boundary between the zones, downwards towards the head of the system, and by the difference of density between the zones.

It is convenient to retain the terms fresh and saline waters because they indicate continuity with their sources. They can be used interchangeably with light and dense in the descriptions. Furthermore, in most small embayments the heat exchange with the atmosphere is uniform over the area, hence the temperature-induced density structure contributes only to the stability, not to the transport mechanism.

The fresh water transport mechanism persists while there is a difference in density between the zones. The entrainment process continually reduces this difference. It may be further reduced by tidal or wind mixing or by loss of fresh water by evaporation or freezing. If it vanishes, the mechanism fails and the waters become homogeneous. Then some of the homogeneous water may be returned to the lower zone of the embayment. Such homogeneity does not normally occur, particularly in embayments where there is no region of complete mixing. Usually there is additional inflow of fresh water along the route of transport.

Tidal Flushing. Tidal currents flood and ebb at all depths to transfer the water necessary for the tidal rise and fall. Within the limits of constancy of mean sea level, the flood and ebb excursions are compensating. There is no net transport of sea water. This does not preclude tidal circulation or tidal flushing, since it is not necessary that the water discharged through a cross-section during the ebb excursion be the same water that entered during the previous flood. The inflow and outflow are usually asymmetric. Hence some part of the tidal volume is renewed during each cycle.

Estuary Mechanism. Usually there are precipitation and land drainage into a marine embayment. Thus there is zone structure, so that both transport and tidal flushing mechanisms occur. In the upper zone the ebb is accelerated and the flood is retarded by the persistent seaward transport. Conversely, the flood is strongest and the ebb is weakest in the lower zone, because of the persistent inward transport. Consequently there is a surface of *No net motion* above which the net transport is seaward and below which it is inward (Pritchard, 1952).

In such cases it is difficult to distinguish between the purely tidal flushing and the transport mechanism. It is convenient to suppose that the tidal excursions do not contribute to the transport, but rather that it is due solely to the fresh water mechanism, that it occurs as a bias on the tidal excursions, and that it is intermittent with the reversing character of the tides.

The Halocline and Entrainment. Turbulence is a consequence of the velocity shear between the upper seaward moving zone and the lower inward moving zone. Turbulence may be regarded as the random exchange of fluid elements. When fluid elements are exchanged vertically in a stratified fluid their displacement is opposed by the stability

$$g \frac{\partial \varrho}{\partial z},$$

where g is gravity, ϱ is density, and z is depth (positive downwards). This acts as a restoring force. The elements tend to return to their original strata. However, having mixed to some extent with surroundings, the densities (salinities) are changed toward intermediate values. Hence they cannot be stable again in their original positions. In effect they must undergo a series of damped oscillations and come to rest in intermediate positions. Thus a halocline is created, in which there is a gradient of salinity from the lower to upper zone.

Entrainment is the preferential upward transfer of lower zone water through the halocline.

It is recognized that fresh water is transferred downward from the upper zone into the halocline. However, if it were transferred below the limit of the halocline, there would be a longitudinal gradient of decreasing salinity toward the head of the embayment in the lower zone. This is not observed. Thus the lower limit of the

halocline must be the limit of downward transfer of fresh or mixed water. On the other hand, it is observed that the upper zone and halocline become progressively more saline to seaward. Hence there must be continual upward transfer of sea water through the lower limit of the halocline. It follows that at this boundary the vertical transfer must be unidirectional, upward.

Above the surface of no net motion the fresh water and entrained sea water are transported persistantly seaward and are eventually lost from the embayment. Fresh water mixed downward to or below this surface must be retarded or transported toward the head of the system. Since it cannot escape from the embayment in these positions and cannot be dissipated in the lower zone, it must remain between the level of no net motion and the limit of the halocline and must be preferentially available for entrainment. It follows that the level of no net motion must be in the halocline.

Thus the necessary entrainment and transition of properties from the lower to the upper zone are achieved. The halocline is the zone of entrainment. The presence of a halocline is sufficient proof that entrainment rather than any other process of upward transfer, such as upwelling (Sverdrup, *et al.*, 1942), is occurring.

Energy of Turbulence. Entrainment depends primarily on the occurrence of turbulent mixing. This can persist only if there is a continuing source of energy. Normally, energy is obtained from the mean flow by the action of turbulent stresses. The intensity of turbulence varies with the energy available in the mean flow, which in turn depends on potential energy or head in the upper and lower zones. La Croix and Tully (1954), in their study of Seymour Narrows, B.C., showed that about 50 % of the available potential energy of the tides was dissipated in turbulence. Evidently, at least part of the energy required for turbulent mixing originates with the tides. This is effective throughout the depth of tidal action, which is usually the whole depth in regions over a continental shelf.

The wind must also be recognized as a source of energy which can lead to turbulent mixing. Eckman (Sverdrup, *et al.*, 1942) showed that the depth (D) of wind influence is

$$D = \frac{6.7\,W}{\sqrt{\sin \Phi}}$$

where W is the wind speed (m/sec) and Φ is the latitude. Within this depth the wind energy is added to the tidal energy, creating further turbulence. All evidence shows that the depth (D) of wind mixing coincides closely with the depth of the nearly homogeneous upper zone. A gradient develops in this layer when the winds are small (Fig. 1, St. A). In the limit, the halocline becomes continuous from the lower zone to the surface.

It is at once evident that the lower limit of the halocline must be determined by the mixing forces. If the depth of the estuary is greater than this limit, the halocline and lower zone waters must be distinct in the structure. This is the case in the deep British Columbia inlets observed by Tully and others (Fig. 1). If, however, the depth of the estuary is less than this limit, the halocline structure must reach the bottom. That is, there must be more or less uniform salinity gradient to the bottom. This was observed by Pritchard (1952) and others studying the plains estuaries on the Atlantic coast of United States.

STRUCTURE

Utilization of these concepts depends on definition of the zones, which in turn requires an accurate description of the structure. This may be accomplished by the method outlined by Tully (1952, 1957). The observed salinity data are plotted as functions of the logarithm of depth (Fig. 2). In this presentation the zones are defined, within small limits of error, by straight lines of constant slope (k) such that

$$S = k\, ln \frac{z}{z_1} + S_1,$$

where z is depth (positive downwards), z_1 is unit depth, and S_1 is a constant.

The first diagram in Fig. 3 shows such a plot of the salinity data from Alberni Inlet, corresponding to Fig. 1. The subsequent diagrams show salinity data from Juan de Fuca Strait.

Examination of more than 1000 examples from regions dominated by fresh water showed that this logarithmic plot could be applied with an apparent error of less than 0.1 $^0/_{00}$ in the halocline and of less than 0.05 $^0/_{00}$ in the lower zone in 90 $^0/_0$ of the cases (Tully, 1957).

The limits of the zones are defined by the intersections of the lines. According to this interpretation of structure there should be

discontinuities at the upper and lower limits of the halocline (D and L). These have not been observed and there is some doubt that they exist. This uncertainty cannot be resolved until adequate instrumentation is available to examine the salinity structure in detail. In the meantime these limits are regarded as "virtual" values.

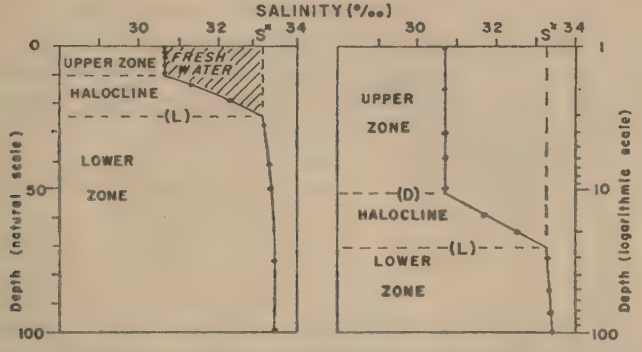

Figure 2. Comparison of natural and logarithmic plots.

The mixing mechanism which creates this structure has not been demonstrated. However, it is intuitively apparent that the mechanism is constant in each zone of constant slope (k). In the halocline this must be the entrainment process.

Index Salinity. The salinity at the lower limit (L) of the halocline defines the water which is about to be entrained. It is called the index salinity (S^*). This has been found to be remarkably constant in any region where the halocline was continuous and where the lower zone water did not change.

Herlinveaux (1959, in preparation), studying Juan de Fuca Strait, and Waldichuk (1957), studying the Strait of Georgia, found that it followed an annual cycle associated with the seasonal variation of sea water intruding the lower zone. Tully and Dodimead (1957) and Dodimead (1958), working in the eastern sub-Arctic Pacific Ocean, found it to be constant (33.8 ± 0.1 ‰) both winter and summer during four years (1955–58), despite major changes in the upper zone. From examination of these data it became evident that index salinity is determined solely by the properties and processes in the lower zone. It is independent of the character of the

upper zone. This confirms the conclusion that the lower limit (L) of the halocline is a surface of unidirectional upward transfer.

Determination of the Fresh Water Fraction (C). From the foregoing discussions, the concentration (C) of fresh water at any position in the upper zone and halocline is

$$C = \frac{S^*L - \int_0^L s\,dz}{S^*L}.$$

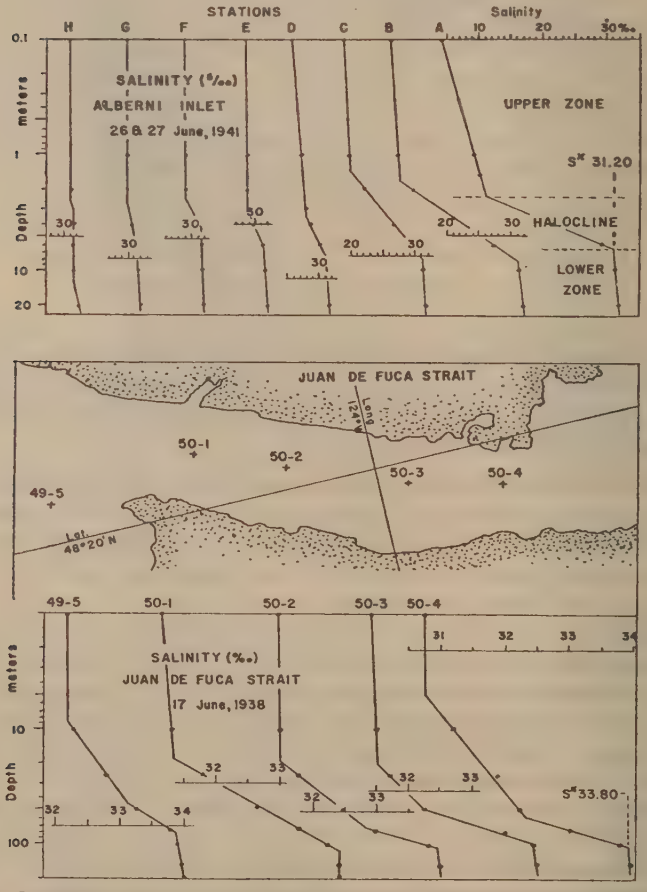

Figure 3. Logarithmic plots of salinity structure in Alberni Inlet and Juan de Fuca Strait, B.C.

The relation is difficult to solve in a natural plot because the structure is often irregular and requires subjective interpretation. Also, the lower limit of the halocline and the index salinity are indefinite. It is more precise and convenient to first plot the salinity data as a function of the logarithm of depth, as has been demonstrated. Here the index salinity (S^*) and the limits of the halocline (D and L) can be accurately determined. With this information a truly representative natural plot can be made. Then the numerator is obtained readily by planimeter integration of the area between the salinity-depth relation and the index salinity. This area is shown shaded in the first diagram of Fig. 2. The denominator is the area expressed by the product of index salinity (S^*) and the depth of limit (L) of the halocline.

TRANSPORT

Fresh water Equations. It has been established that all fresh water together with the entrained sea water is transported seaward through the upper zone and halocline. Then to maintain continuity the fresh water fraction (C) of the total volume transport through any cross-section of the upper zone and halocline (T') must equal the rate of inflow of fresh water (Q) from the head to that section. Taking the positive direction seaward

$$C T' = Q, \tag{1}$$

whence the total transport through the upper zone and halocline is

$$T' = \frac{Q}{C} \tag{2}$$

in units of volume per unit time. Because of the diurnal cycle of the tides it is implied that the unit of time is the tidal day (24 hours, 52.2 minutes) regardless of the units used in calculation.

It is also evident that the seaward transport of the sea water fraction ($1 - C$) of the upper zone and halocline must equal the upward transport ($-W'$) through the interzone boundary in the area between the section and the head of the embayment. This in turn must equal the inward transport through the cross-section in the lower zone (T''), else sea water would accumulate or decrease in the embayment

$$-W = T'' = -(1-C)T' = \frac{1-C}{C}Q. \tag{3}$$

It is stipulated in these equations that the zones are defined in the structure (Fig. 2) and that transport is primarily dependent on the fresh water inflow (Q). This is the total of precipitation, river discharge, and seepage, inwards of the cross-section being considered. These quantities vary considerably and are difficult or impossible to measure accurately. Hence it is necessary to introduce another concept to solve the equations.

Transverse Forces. During the flooding tide, the maximum velocity is in the lower zone. In the northern hemisphere these waters must tend to the right more than those in the upper zone. Hence the greatest depth of lower zone (dense) water must be on the right of the inward flow. Conversely, during the ebbing tide the greatest velocity is in the upper zone. These waters must tend to the right more than those in the lower zone. Thus the greatest depth of upper zone (light) water must be on the right of the seaward flow.

In a situation where the flood and ebb flows are opposite, there must be a cross-channel slope of the isopycnal surfaces, representing the gravity forces required to balance the Coriolis forces which are associated with the difference of velocity between the upper and lower zones. This is the case in Juan de Fuca Strait, illustrated in Fig. 4. Then

$$\frac{d_2-d_1}{\Delta y f} = \bar{V}' - \bar{V}'', \tag{4}$$

where d_1 and d_2 are the anomalies of dynamic height across the channel, Δy is the width, f is the Coriolis parameter ($2\omega \sin \Phi$), and $\bar{V}'$ and $\bar{V}''$ are the mean velocities in upper and lower zones.

The velocity difference can be related to the transports in

$$\bar{V}' - \bar{V}'' = \frac{T'}{A'}\frac{T''}{A''}, \tag{5}$$

where A' and A'' are the cross-sectional areas of the upper and lower zones which can be defined from the structure.

Transport. Combining the concepts it is possible to evaluate the transport.

Substituting for T'' from eq. (3),

$$\frac{d_2-d_1}{\Delta yf} = \frac{T'}{A'} + \frac{1-C}{A''} T',$$

whence the seaward transport in the upper zone is

$$T' = \frac{d_2-d_1}{\Delta yf} \cdot \frac{1}{\frac{1}{A'} + \frac{1-C}{A''}} \tag{6}$$

and the inward transport in the lower zone is

$$T'' = -\frac{d_2-d_1}{\Delta yf} \cdot \frac{1}{\frac{1}{A'(1-C)} + \frac{1}{A''}}. \tag{7}$$

In eqs. (6) and (7) all terms on the right side of the equations can be accurately determined in normal oceanographic data.

The application of these equations is demonstrated in a study of Juan de Fuca Strait (Tully and Herlinveaux, 1960).

Comments. Cameron (1951) observed the cross channel slope of the isopycnal surfaces in Portland Inlet, B.C. He showed that the difference of velocity between the upper and lower zones agreed well with that deduced from direct current observations. Pritchard (1956) made further studies in the James River. He observed that the lateral gradient of pressure is not exactly balanced by the Coriolis force, as calculated from the observed mean flow. He ascribed the residual force to the gradient of a lateral Reynold's shear stress. Stewart (1957) showed that the discrepancy was probably due to curvature of the flow path.

These studies show that the technique is applicable if care is taken to choose a cross-section in a straight reach of the embayment, or where curvature of the flow path is not a consequence of boundary limitations.

In many cases the velocity-depth gradient remains nearly constant throughout the cycles of tidal flow, as in Juan de Fuca Strait (Fig. 4). In such cases the transverse slope of the isopycnals must remain almost constant. Hence any measurement of the difference of dynamic heights may be utilized, regardless of the stage of the tide during observations. However, in those cases where the velocity gradient, and hence the slope of the isopycnals, varies with the tide, it is implied that the mean values through a tidal day should be used in the equations.

The dynamic heights may be evaluated from any columns in the section which extend to or below the deepest level of the interzone boundary (L). It is unlikely that there would be significant differences of dynamic heights below this level.

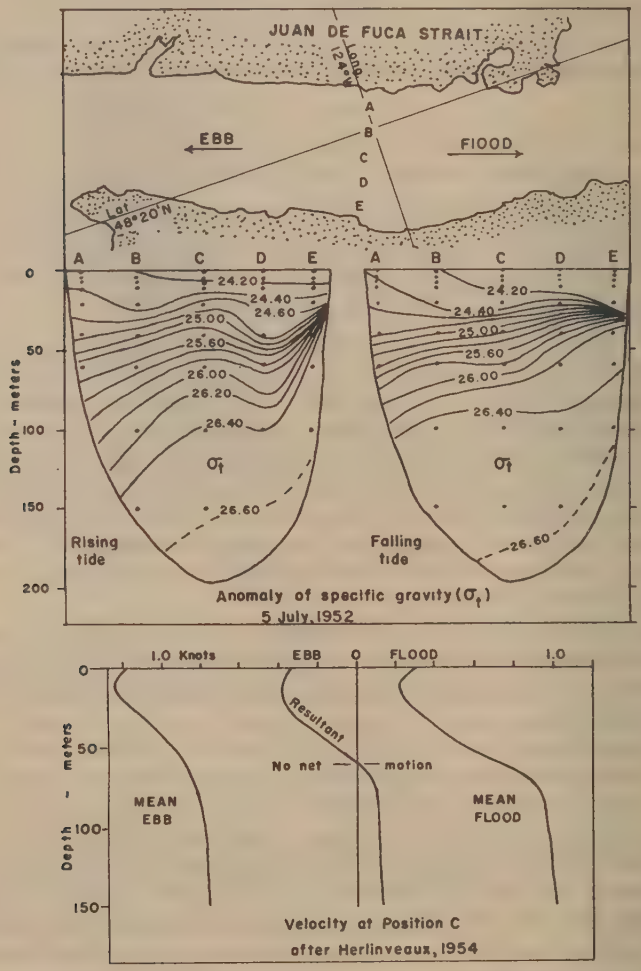

Figure 4. Density and velocity structure in Juan de Fuca Strait, B.C.

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SOME OCEANOGRAPHIC CHARACTERISTICS OF A POLLUTED INLET IN BRITISH COLUMBIA

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REPRINT FROM JOURNAL OF MARINE RESEARCH, VOLUME 17. 1958

FRB No. 643

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ABSTRACT

Physical and chemical characteristics of Neroutsos Inlet, British Columbia, in August 1957 are described. Moderately deep, 11 nautical miles long by 0.5 to 1 mile wide, it has been subjected to a prolonged period of waste disposal from a sulphite pulp mill near its head. The mill effluent is discharged on the surface at the shore and is largely confined to a thin surface layer about 5 m deep. Low dissolved oxygen in surface water was noted and virtual extinction of intertidal flora and fauna within several miles of the mill apparently has occurred. Absence of significant vertical mixing allows the deep water to remain relatively free of contamination. The Inlet lacks the mechanism of a large runoff from the head for rapid flushing of wastes released at the surface.

Comparison of oceanographic data collected in Neroutsos Inlet during August 1927 with those of August 1957 suggests that there has been a long-term change in conditions as a result of 40 years of continuous discharge of mill wastes into Inlet waters.

INTRODUCTION

Neroutsos Inlet is the southeast arm of Quatsino Sound, on the northwest side of Vancouver Island (Fig. 1). It has a northwest-southeast axis parallel to the main trend of the coastline, running from Lat. 50°20′ N to 50°30′ N. Shaped like a typical British Columbia fjord (Pickard, 1956), it is long and narrow with steep mountainous sides. It is 11 nautical miles long, divided into a deep section $7^1/_2$ mi long and 1 mi wide from its mouth to Ker Point and a shallow section $3^1/_2$ mi long and $^1/_2$ mi wide from Ker Point to its head. A cross-section of the Inlet is U-shaped with depths slightly greater than 100 fathoms along midchannel. It has no significant sills, but it is somewhat constricted laterally and vertically at Ker Point, where the effective width is less than 0.3 mi and the depth is only 20 fathoms. In Table I are given some of the morphometric characteristics of the Inlet.

Figure 1. Chart of Neroutsos Inlet and adjoining waters of Quatsino Sound showing oceanographic stations occupied during August 1957. *Inset*: Chart of Vancouver Island featuring the general location of Quatsino Sound.

TABLE I. Morphometric Characteristics of Neroutsos Inlet

Dimension	*Common Units*	*Metric Units*
Length from mouth at Pender Pt. to Ker Pt.	7.70 n mi	14.27 km
Length from Ker Pt. to head at Cayeghle Cr.	3.50 n mi	6.49 km
Average width of segment from Pender Pt. to Ker Pt.	0.91 n mi	1.69 km
Average width of segment from Ker Pt. to Cayeghle Cr.	0.47 n mi	0.87 km
Mean depth	48.1 fm	88.0 m
Mean large tidal range	9.5 ft	2.90 m
Mean small tidal range	6.0 ft	1.83 m
Mean tidal prism on large tidal range	0.0140 (n mi)^3	0.0890 km^3
Mean tidal prism on small tidal range	0.00886 (n mi)^3	0.0563 km^3

Horizontal areas:

Depth Contour	*Area* (n mi)^2	*Area* km^2	*Depth Contour*	*Area* (n mi)^2	*Area* km^2
Surface*	8.944	30.77	50 fathoms	4.022	13.84
10 fathoms	6.990	24.05	60	3.469	11.93
20	6.285	21.62	75	2.708	9.315
30	5.146	17.70	90	1.822	6.268
40	4.631	15.93	100	1.076	3.702

* Mean high water.

Cross-sectional areas:

Section	*Area* (n mi)^2	*Area* km^2
From Pender Pt. to point west of Atkins Cove	0.0448	0.1540
From Nequiltpaalis Cr. to Goquawhenigh Cr.	0.0726	0.2495
From Port Alice to Cayuse Cr.	0.00393	0.01348
Across Inlet south of Ketchen Is.	0.00976	0.0335

Volumes:

Depth Interval (fathoms)	*Volume* (n mi)^3	*Volume* km^3	*Depth Interval (fathoms)*	*Volume* (n mi)^3	*Volume* km^3
0–10	0.0761	0.4838	60–70	0.0316	0.2007
10–20	0.0653	0.4153	70–80	0.0267	0.1699
20–30	0.0568	0.3612	80–90	0.0213	0.1353
30–40	0.0483	0.3070	90–100	0.0143	0.0912
40–50	0.0425	0.2706	100–110	0.0055	0.0352
50–60	0.0367	0.2334	Total	0.4251	2.7036

Like most British Columbia inlets, it has a tributary stream at the head. Cayeghle Creek forms a silty delta at the southern end of the Inlet where winter freshets deposit most of the load eroded

from the rugged drainage basin. Its discharge is of the order of 50 cfs (1.4 m^3/sec) in midsummer, with much greater flows in winter when heavy rains give rise to a large runoff. Numerous streams dissect its margin but they contribute relatively small volumes of fresh water to the system. Compared to other B.C. inlets, the total volume of fresh water added to Neroutsos Inlet by runoff is small because of the confined area of the drainage basin.

The steep slopes bordering this Inlet are heavily wooded. This makes the area attractive for logging, and forest products industries have been established there from early times.

Meteorological conditions in Neroutsos Inlet are typically those of the west coast of Vancouver Island. Summers are moderately warm and dry, but a large winter rainfall augmented by occasional snow gives a mean annual precipitation of 115 inches (292 cm), measured at Port Alice. While the Inlet is sheltered from intense Pacific storms, it has its own wind regime, which can be quite intensive at times. Typical of B.C. inlets, there are strong up-inlet winds (northwesterly in this case) on warm sunny afternoons. During inclement weather, winds are southeasterly or down-inlet.

POLLUTION SOURCE

At Port Alice, on the east side of the upper section of the Inlet, a sulphite pulp mill has been in operation since 1917. Its production has varied from somewhat modest beginnings through a shutdown period of many months during the 30's to about 220 short tons per day by 1942. Output has continued into 1959 at the latter figure, excepting a few months of supension of production in 1957–58. In 1955, construction was commenced on an expansion of the mill to increase production by two thirds to approximately 370 tons per day (Waldichuk, 1956a).

This mill employs a calcium base in its cooking process, and wastes pass untreated into the Inlet. Discharge of effluent, until 1957, was by way of a wooden flume. The outfall from which the wastes were released was about 15 ft above lower low tide, providing considerable mixing of effluent into the surface water. In 1957, a specially designed flume was installed to allow the effluent to discharge into the surface water with a minimum of mixing. The effluent from the mill has a flow of about 29,000 U.S. gallons per

minute (65 cfs, or 1.84 m^3/sec), the water for which is piped from Victoria Lake, located a few miles east of Port Alice.

For every ton of pulp produced, one ton of organic substances that bind the wood fibers together, in addition to about 800 lb of chemicals used in the digestion, are passed into the receiving waters. The main cause of pollution is the high oxygen consumption during decomposition of the organic constituents. With an average of 650 lb of BOD (biochemical oxygen demand) per ton of sulphite pulp (Gehm, 1953: 203), the oxygen demand for a daily output of 220 tons of pulp would be 14.3×10^4 lb (6.49×10^4 kg).

METHODS AND DISTRIBUTION OF PROPERTIES

A survey was conducted in Quatsino Sound August 15–20 with the ship CNAV EHKOLI. Regular oceanographic observations of salinity, temperature, dissolved oxygen and pH were made at stations shown in Fig. 1. In addition, water samples were analyzed for concentration of spent sulphite liquor (SSL). Water samples were taken with a bucket from the surface and with Nansen sampling bottles from depths of 1.8, 4.6, 9.1, 13.7, 18.3, 27.4, 45.7, 91.4, and 137.2 m (6, 15, 30, 45, 60, 90, 150, 300, and 450 ft) or from as many of these levels as the depth of water would allow.

Samples for salinity were taken in 8-oz (237 ml) medicine bottles that were corked securely and waxed to prevent evaporation. Titration for salinity was carried out at the base laboratory by the standard Mohr method.

Dissolved oxygen samples were drawn into 300-ml BOD bottles and treated for titration immediately on board ship. The standard Winkler procedure set up in a shipboard oxygen titration kit was used. The kit allowed 100-ml aliquots of treated sample to be drawn with an automatic pipette attached to a vacuum system, and agitation during titration was effected with a magnetic stirrer. Standardization of sodium thiosulphate was carried out with a standard solution of 0.01 N potassium bi-iodate.

Hydrogen-ion concentration was determined on shipboard using a Beckman pH meter (Model H-2, 110 volts a.c.). All samples were brought to laboratory temperature in a water bath and the pH readings observed were corrected for temperature of the water samples *in situ*.

Concentrations of spent sulphite liquor were determined at the base laboratory on the samples used for salinity titration. Samples of 50 ml were treated according to the method described by Pearl and Benson (1940). A Beckman DU Spectrophotometer set at 410 mμ wavelength was used for absorption measurements. Undiluted digestor liquor from the pulp mill was used as the standard; concentrations of SSL in the water samples are given as parts of the digestor liquor per million parts of water (ppm). Naturally occurring lignin-like substances in inshore sea waters contribute apparent SSL concentrations of the order of 5 ppm. Hence, it is considered that any values lower than 10 ppm are insignificant insofar as contribution from the pulp mill is concerned.

Distributions of conservative properties, shown in Fig. 2, are typical of a stratified inlet. The salinity distribution indicates a relatively high salt content, almost 33 $^0/_{00}$, in the deep water; compared to the deep water in Alberni Inlet (Waldichuk, 1956b), this is about 0.5 $^0/_{00}$ higher. The surface water exhibits moderately low salinities, which are confined primarily to the head of the Inlet. A marked salinity stratification in the upper 5 m is apparent near the head where salinity ranges from 13 to 25 $^0/_{00}$ in a shallow surface layer. Variation of salinity with depth is less obvious near the mouth where the range is only 2.5 $^0/_{00}$, from 30.5 $^0/_{00}$ at the surface to 33.0 $^0/_{00}$ near bottom.

Temperatures observed during the survey showed the effect of surface warming of the water. The vertical gradient found at the head of the Inlet (surface 17°, bottom 8° C) diminishes seaward and is markedly less at the mouth of the Inlet (surface 15°, bottom 8° C). A certain amount of mixing evidently takes place near the mouth which partially destroys thermal or salinity stratification set up locally. Tidal movement from the relatively well-mixed areas of Quatsino Sound brings water into Neroutsos Inlet which is more homogeneous than that formed therein.

The density (σ_t) distribution exhibits the large range resulting from salinity and temperature gradients, both tending at this time to make the surface water light and the deep water dense. A large range in σ_t from 8.5 at the surface to 23 at a depth of 10 m in the upper 10 m near the head of the Inlet exemplifies the great stability of this surface layer.

The nonconservative properties (Fig. 3), especially dissolved oxygen concentration, exhibited distributions which were unusual

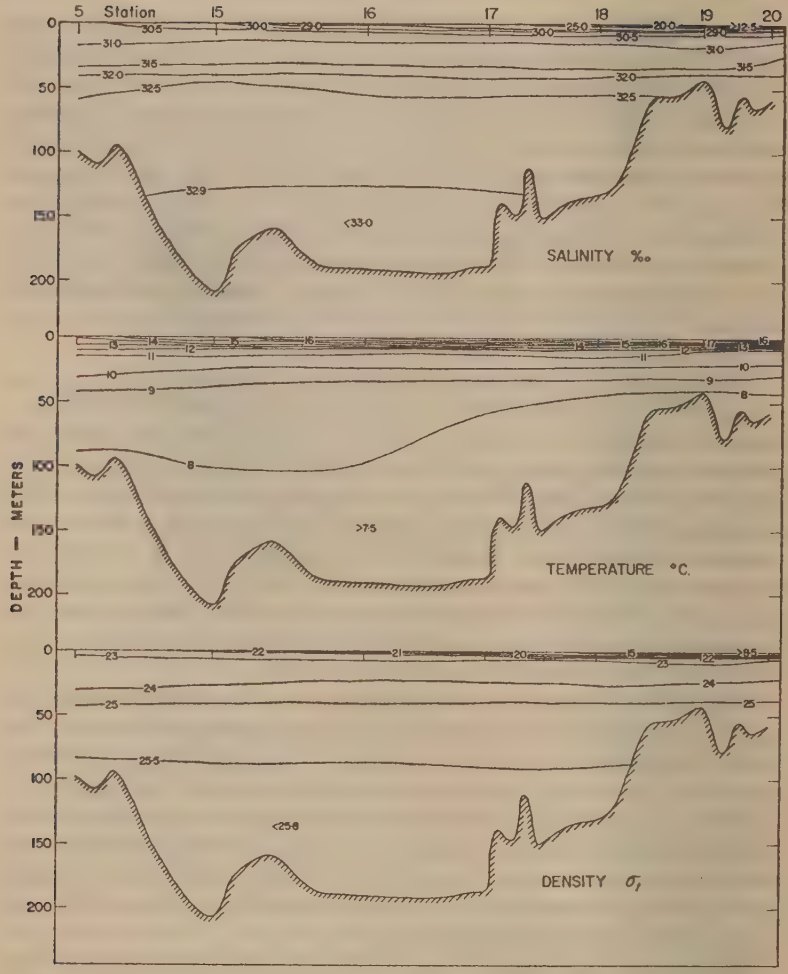

Figure 2. Distributions of salinity, temperature, and density (σ_t) in longitudinal sections through Neroutsos Inlet, August 1957.

for a normal inlet but which are probably typical for one receiving wastes from a sulphite pulp mill. Surface dissolved oxygen concentrations in the upper part of Neroutsos Inlet, within a radius of a mile of the mill, were as low as 0.16 mg-at/l; up to 2 mi north and

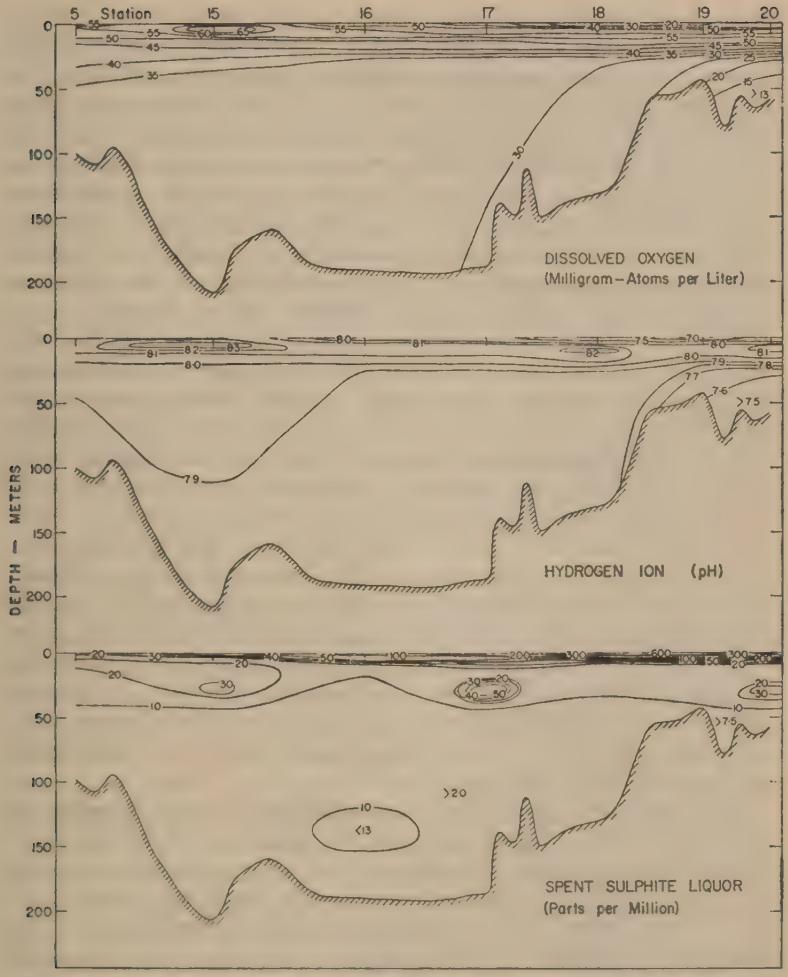

Figure 3. Distributions of dissolved oxygen, pH, and spent sulphite liquor in longitudinal sections through Neroutsos Inlet, August 1957.

south of the pulp mill they were less than 0.3 mg-at/l. This layer of low dissolved oxygen was relatively shallow, less than 5 m deep. Below the surface layer, concentrations of dissolved oxygen rapidly increased to a maximum, greater than 0.55 mg-at/l at a depth of

about 10 m. Whether this high oxygen content resulted from "fresh" sea water moving up the Inlet under the seaward-moving surface layer or from high local photosynthetic activity is uncertain, but the latter cause is suspected. Below this high oxygen zone there was a decrease in dissolved oxygen until concentrations as low as 0.15 mg-at/l were reached in the bottom water at the head of the Inlet. Low oxygen concentrations in this part may be partially due to decomposition of organic substances released from the mill. However, it is probable that low dissolved oxygen is a natural consequence of horizontal and vertical constriction of the Inlet near Port Alice and of a lack of vertical flushing behind the sill.

Dissolved oxygen concentrations in the lower part of the Inlet were typical of ventilated British Columbia inlets. A moderately high dissolved oxygen concentration at the surface increased to supersaturation in a zone at about 5 m depth where vigorous photosynthesis was apparently occurring. In the deep water there was a gradual decline in the amount of dissolved oxygen down to the bottom, where concentrations were just over .30 mg-at/l.

The distribution of pH was closely parallel to the distribution of dissolved oxygen, but variations of pH were not as large. Except for the area in the vicinity of the mill, where surface pH was just under 7.0, the sea water exhibited a high of about 8.0 at the surface and a low of 7.6 at depth. The region of oxygen supersaturation near the surface at St. 15 was characterized also by a high pH, greater than 8.3. Deep water in the upper Inlet showed a relatively acidic state with a pH of less than 7.6, but that in the lower Inlet was comparatively alkaline, 7.8 to 7.9.

Spent sulphite liquor in high concentrations of over 200 ppm was confined to a thin surface layer of less than 5 m thickness. These concentrations extended for about 2 mi north and 1 mi south of Port Alice. There was a rapid decrease of SSL with depth in all parts of the Inlet. Cells of relatively high concentration of SSL found at intermediate depths suggest that there was occasional mixing of effluent with deep water either through wind or tidal action. Concentrations of less than 10 ppm shown in the deep water may be unrelated to the pulp mill waste since they could be caused by naturally occurring lignin-like substances in sea water.

OTHER OBSERVATIONS

Color of the water was a reddish brown from the head of the Inlet to about 5 mi below the mill. As measured with a Secchi disc, the depth of visibility increased from about 2 m in midchannel opposite the mill to 5 m at a distance of 6 mi from the mill, to 6 m at the mouth of the Inlet and to 7 m at the entrance to Quatsino Sound. According to reports from aqualung divers, visibility in the water in Port Alice Harbor is poor to a depth of 4 or 5 m; below this depth, even adjacent to the mill, the water is generally clear and visibility is good, with sufficient light penetrating through the dark upper layer to allow moderate illumination.

A considerable amount of foam was observed in the Inlet, particularly on windy days. The densest foam was in the immediate vicinity of the outfall, but wind-stirring produced streaks of foam paralleling the direction of the wind for considerable distances down the Inlet. Presence of foam is a direct consequence of the presence of SSL, which has a low surface tension and tends to froth when aerated by stirring.

While no quantitative biological sampling was carried out, some incidental observations of fish, intertidal fauna, and vegetation were made. For a mile north and south of Port Alice on both sides of the Inlet, no evidence could be found of intertidal plant or animal life, excepting a greyish-green fungus slime, possibly *Sphaerotilus*, which is characteristic of pollution from SSL (Neale, 1958). The cobbles, sand, and other shore materials were plated with a glossy, pitch-like substance. Presence of molluscan shells in the intertidal zone suggests that intertidal animals were common at one time. The pilings of the docks and other waterfront structures at Port Alice were remarkably free of any fouling organisms. It has been remarked by local residents that a good way to remove fouling from boats is to tie up at the mill's wharf for a few days.

While night observations in the presence of a deck lamp secured on the ship usually provided an opportunity to observe a large number of small fish swimming into the illuminated area around the ship, no fish, large or small, were observed at a 30-hour anchor station in Port Alice Harbor. Only two jellyfish were noted drifting past the ship during daylight observations.

About 1.5 mi north of Port Alice, at Jakopaal Creek, particularly where flushing by fresh water occurs, a few barnacles and some

seaweed were noted on the rocks. At the head of Neroutsos Inlet, where Cayeghle Creek enters over the tide flats, 2.5 mi south of Port Alice, occasional small mussels in addition to sparsely populated barnacles on old pilings were found. On the shores around Frigon Island, 2.3 mi north of Port Alice, a more normal distribution of plant and animal life than that to the south was found. Sea weed was present in great profusion on the rocks, barnacles in fairly large concentrations encrusted shore materials of the intertidal zone, and the water in this region was considerably clearer than that found only a mile to the south.

CHANGE IN OCEANOGRAPHIC CONDITIONS OF NEROUTSOS INLET FROM 1927 TO 1957

The first oceanographic observations conducted in Neroutsos Inlet to note the effects of the Port Alice pulp mill were made on 17 August 1927 by Hutchinson and Lucas (1927) on the CGS MALASPINA of the Federal Fisheries Department. This was 10 years after commencement of pulp production at Port Alice and exactly 30 years prior to the August 1957 survey. The 1927 observations were initiated in consequence of complaints of alleged harmful effects of the wastes from the mill upon the fishing industry.

Five stations were occupied at distances of 0.01, 1.25, 3.5, 7.5 and 15 n mi seaward from the mill. Distributions of salinity and temperature in deep water were not especially different from those observed in 1957. However, in the upper 10 m, salinities were greater by 1 $^0/_{00}$ in 1927 than in 1957. At the head of the Inlet, the difference in surface salinity was more than 10 $^0/_{00}$, the lower salinity occurring in 1957. Temperatures in the upper 5 m were also higher by 1 to 4° C during the 1957 survey.

Dissolved oxygen concentrations observed during August 1927 and August 1957 were significantly different, especially in the surface layer. In Fig. 4, profiles of vertical oxygen distribution for the stations occupied in 1927 are compared to those 1957 stations located nearest to them. There were low dissolved oxygen concentrations at the surface at stations near the pulp mill in 1957; these were not apparent in 1927. At 10 m, the oxygen concentrations were considerably higher at all stations in 1957 than in 1927. The total amount of dissolved oxygen between 10 m and the surface was greater in 1957 than in 1927 at the two seaward stations only

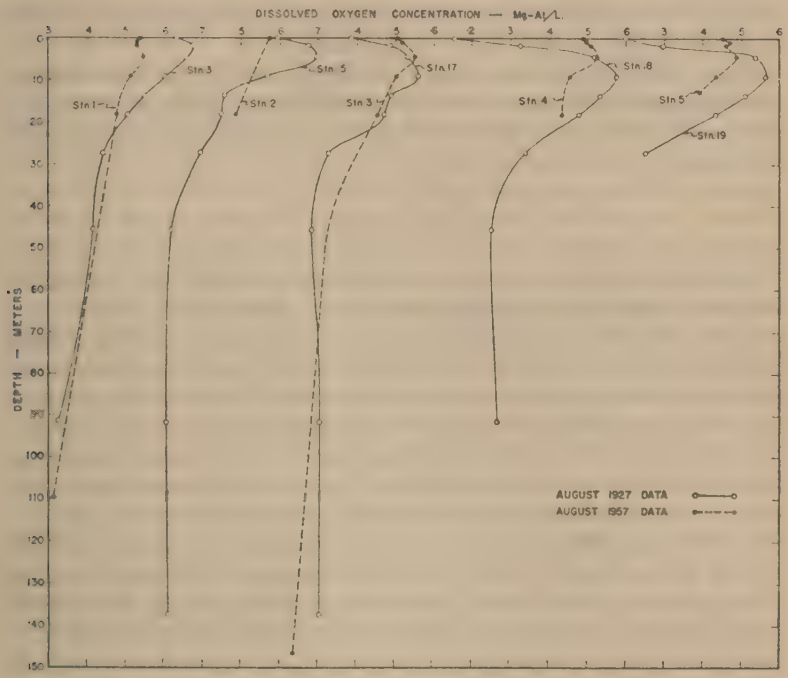

Figure 4. Profiles of dissolved oxygen distribution at stations of comparable location in Quatsino Sound during August 1927 and August 1957.

(Sts. 3 and 15 for 1957; Sts. 1 and 2 for 1927). From the available data, the dissolved oxygen concentrations in deep water appeared to be about the same during both survey periods.

Plankton observations carried out in 1927 showed the presence of various forms of phytoplankton and zooplankton which are expected normally in sea water during the season when samples were taken. It was stated by the authors that plankton organisms were not found in the immense quantities which characterize areas where pilchard were abundant. The fishery guardian reported, at the time, that salmon were being caught in Neroutsos Inlet as formerly. Herring were abundant and spawned in the Inlet during the season prior to August 1927; some dead herring were found on shore, but the cause of death was not known. A pilchard "run" to the head of

Neroutsos Inlet had occurred in 1925 but had not recurred in 1926 or in 1927 prior to the time of the survey.

Hutchinson and Lucas (1927) concluded "that the chemical and physical properties of the water in South East Arm, Quatsino Sound, were well within the limits of sea waters of the continental shelf at the time of this investigation."

DISCUSSION

The low dissolved oxygen concentrations found in the surface waters of Neroutsos Inlet appear to be a direct result of the spent sulphite liquor discharged from the Port Alice pulp mill. The weather prior to the 1957 survey was probably such as to accentuate pollution characteristics in the surface water. That is, a period of abnormal precipitation and runoff was followed by calm warm weather that allowed stratification of surface water to take place with little mixing of effluent into deep water. High surface temperatures promoted decomposition of the mill wastes. Conditions observed at this time were probably the worst for the year, and it is likely that dissolved oxygen concentrations are near normal in the surface water during autumn and winter periods when wind mixing is common.

Because of the geography of Neroutsos Inlet, the dispersion of industrial wastes is confined to essentially one dimension, along the axis of the Inlet. In this respect, an inlet is probably the marine feature most comparable to a land river. It differs widely from other coastal features such as a straight open shoreline or a bay, where dispersion of waste effluent can occur in at least two dimensions. For this reason, high concentrations of SSL can be found at greater distances from the source in Neroutsos Inlet than in an area such as Port Gardner Bay (Eldridge and Orlob, 1951).

That pulp mill pollution has affected the intertidal fauna in Neroutsos Inlet there is little doubt. The effects on fish are more obscure, since fish are able to survive in the deep oxygen-rich water. There are reports of fish, mainly rock cod, having been taken near the wharf at Port Alice in the vicinity of the mill sewer outfall. Divers working in waters adjacent to the mill have noted large numbers of sea-perch, rock cod, and other bottom-dwelling fish in the deep water. Occasionally there are reports of dead rockfish being found in the inlet, as during July 1955, when a number of these were collected during their last stages of life by the Fisheries

Patrol Vessel PHOLIS following a very low tide after a week of dry weather.

Small salmon runs are regularly recorded by Fisheries inspectors in streams at the head of the Inlet. It is presumed that these fish frequent or pass through the water layers below 5 m depth, where the oxygen supply is ample and where concentration of SSL is small. Downstream migration of salmon fingerlings is probably accompanied by heavy mortality inasmuch as juvenile salmon tend to swim along the surface and linger in embayments during their seaward migration. Their destruction would be unnoticed, since small salmon tend to sink when dead. It has been suggested (Wickett, personal communication) that pink salmon, *Oncorhynchus gorbuscha*, do not spawn in the rivers tributary to Neroutsos Inlet since juveniles of this species are small when they enter the sea and are sensitive to pollution. Since runs of pinks normally occur in other inlets of the Quatsino Sound system, it is reasonable to assume that they probably cannot withstand the effect of sulphite waste in Neroutsos Inlet. Fisheries inspectors examining Cayeghle Creek and its tributary, Colonial Creek, have reported that they are favorable streams for salmon spawning but are underutilized. At present, only silvers (*O. kisutch*), chinook (*O. tshawytscha*) and dog (*O. keta*) salmon, which produce much hardier downstream migrants than pinks, spawn in the streams. In the opinion of the Fisheries inspectors, Neroutsos Inlet is by far the most inferior inlet of the Quatsino Sound system as a salmon producer.

It is curious that the chemical and biological properties of the water in this Inlet were so different in 1957 from those observed in 1927. It is true that in 1927 the mill was not operating at its present capacity of 220 tons per day. Furthermore, weather conditions preceding the 1927 survey probably differed considerably from those preceding the recent survey. Most of the 1957 stations were occupied during the early morning when the water was calm and when there was no aeration by wind mixing. However, these differences do not seem to be sufficient to explain the large differences in characteristics observed.

The change in environment due to adverse conditions created by pollution is not always a sudden sort of thing. Deterioration may be a gradual process. Evidently, it is partially this type of decay which has occurred in Neroutsos Inlet. Ten years after the mill commenced operation, chemical and biological observations of the water indi-

cated little pollutional effect from the pulp mill, and biological characteristics appeared to be normal. However, 40 years after the mill went into operation, a marked change has been noted.

Neroutsos Inlet, unlike Alberni Inlet (Tully, 1949), does not possess freshwater characteristics which would provide adequate flushing of surface waters. Because runoff from the head of the Inlet is small, there is not the large seaward flow at the surface that is present in most other inlets. Consequently, flushing of effluent must rely on the small existing stream inflows in addition to displacement by the effluent itself. There is a small net daily displacement seaward as the effluent moves back and forth with the tide. Flushing efficiency in Neroutsos Inlet is relatively small compared to that in other British Columbia inlets. With the absence of deep vertical mixing, the effluent is confined to a relatively shallow surface layer.

ACKNOWLEDGMENTS

It is a pleasure to record this contribution in honour of Professor Thomas G. Thompson, under whose guidance the author had the privilege of studying oceanography. Alaska Pine and Cellulose Limited at Port Alice provided basic information on their pulp production and supplied digestor liquor samples for standards in analysis of sea water for SSL. Their co-operation is gratefully acknowledged. Unpublished data collected in Quatsino Sound by Dr. A. H. Hutchinson and Mr. C. C. Lucas during 1927 have been used here through the courtesy of the Department of Fisheries in Vancouver, where their report was held on file.

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The Study of In-Situ Marine Photosynthesis Using a Large Plastic Bag

Any precise study of the processes of phytoplankton growth and decay in the sea is impossible unless the same body of water is analysed over a protracted period of time. This necessitates some form of containment but the use of a tank has several disadvantages. Unless the tank is very deep the lighting of the water mass as a whole is unrepresentative of that found in the euphotic zone and the degree to which water in the tank is heated will generally be inadmissibly high. Finally no "balance" of dissolved oxygen or carbon dioxide can be readily computed using a shallow body of water with a large surface area in contact with the atmosphere.

We wish to report the construction of a piece of equipment the use of which has overcome the above disadvantages and which has enabled a study to be made of the growth and decay of marine phytoplankton under near "natural" conditions.

The equipment consisted of a thin plastic container connected to the atmosphere by a narrow neck, the body of the apparatus being some 6 ft beneath the surface of the sea so that much of the sun's heat was removed before reaching the container. To lessen the effects of wall growth and accidental contamination both the volume and the volume to surface area ratio of any container should be made as large as practicable. These facts and considerations of ease of construction, stirring and sampling suggested the use of a spherical bag at least 20 ft in diameter. In coastal waters it is doubtful whether increasing the size of a bag by more than another 5–10 ft would have any advantage as for much of the time the bottom would then be beneath the euphotic zone.

The apparatus is shown in plan and to scale by Figure 1. A custom-made bag made of 8-gauge opalescent polyvinyl chloride sheet was used. The bag was exactly 20 ft in diameter, with an 18-in. diameter neck some 10 ft long and was supported by 6½-in. mesh netting of number 12 nylon seine twine. About 80–85% of the incident light was transmitted by this assembly. The netting was fastened to steel rings 10 or 20 ft in diameter, as shown, and the whole attached, by a steel "cradle," to a fiberglas buoyancy bell. The latter could be flooded with water or filled with air to counteract any increase or decrease in weight of the system brought about by salinity changes in the water surrounding the apparatus. (These changes are very considerable in British Columbia Coastal waters in the spring and a possible weight increase of up to 4000 lb had to be tolerated by the system). Weights were tied to the bottom ring of the apparatus after it was filled to ensure that no upthrust could develop on the buoy. The level of the buoy, and hence the bag, beneath the surface of the sea, was kept constant by adding or removing air as necessary (generally not more frequently than once a day).

The neck of the plastic bag was threaded up through the buoyancy bell and tied to the top. A fiberglas liner was then pushed down the inside of the neck and projected a few feet into the bag. This prevented the plastic from closing when density gradients in the water inside and outside the neck differed and it also lessened wear-and-tear on the plastic neck during sampling. At the end of an experiment the bag could be replaced without removing the whole apparatus. The bottom 10-ft diameter ring was removed, the old bag pulled through by SCUBA divers and a new bag, suitably folded, threaded through the neck of the buoy.

The whole equipment was anchored to three one-ton anchor blocks by three nylon ropes, about 150 ft long, fastened to halters on the central 20-ft diameter ring. The bag was thus at the center of an isosceles triangle, with the anchors at the three points, and was only able to move laterally within a circle of 10-ft or less radius.

The contents of the bag were kept stirred

Reprinted from Limnology and Oceanography, Vol. 6, No. 1, pp. 93–96, January, 1961.

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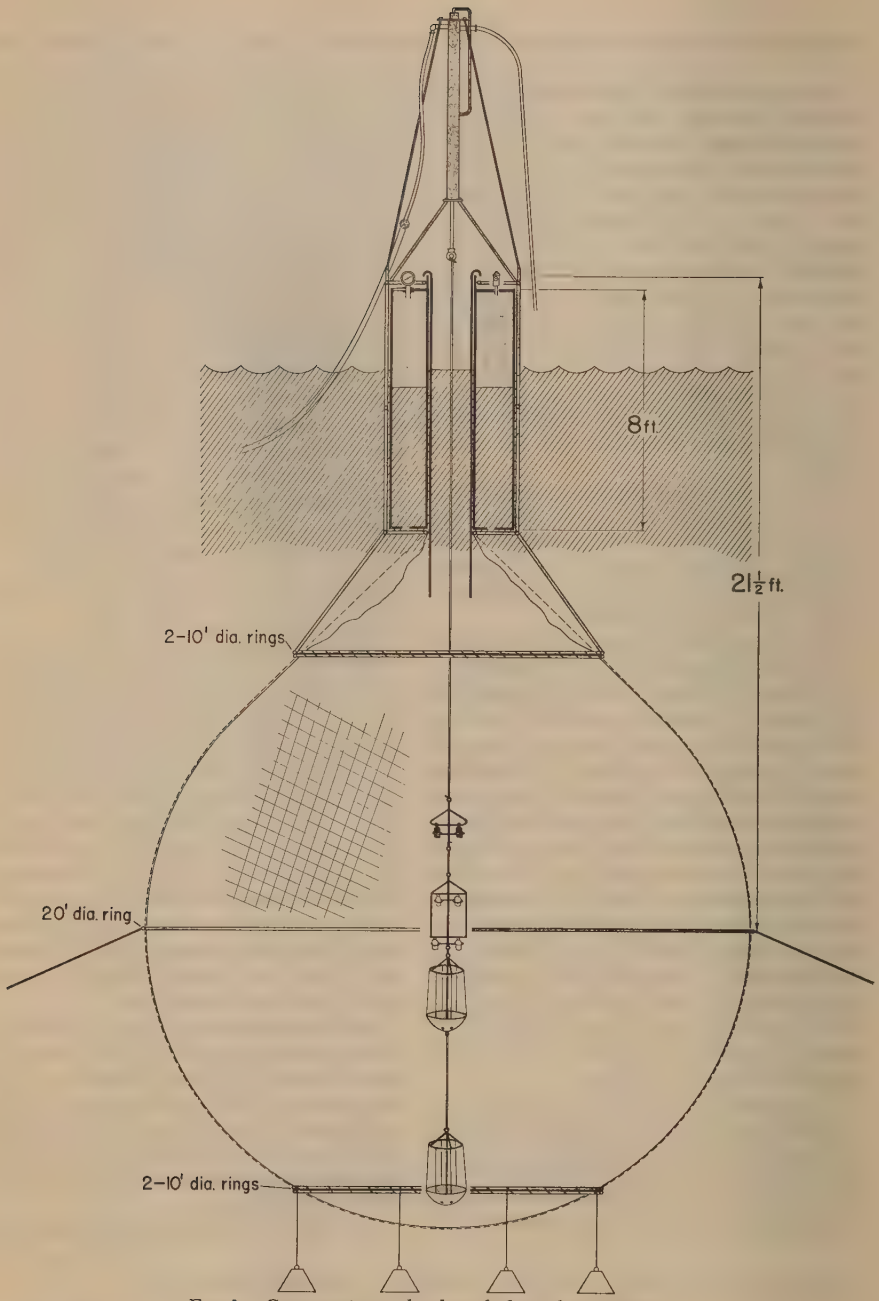

Fig. 1. Cross-section scale plan of plastic bag container.

to uniformity to enable an accurate analytical balance of particulate and soluble material to be evaluated. The stirring was accomplished by an umbrella-like device, made of nylon and stainless steel, which opened on the up-stroke of a piston having a length of 2½–3 ft. An alternative arrangement with two "umbrellas," one at the top and one at the bottom of the bag, connected by rigid rod was also tried, the umbrellas being upside down so that they pushed water downwards. This latter system should, theoretically, give a greater upward velocity to water in the bottom part of the bag and hence better counteract the sinking of plant cells but there may be little to choose between the two arrangements.

The stirring motor, suspended on the buoyancy bell by a quadraped, was water driven from a pipe from the shore. It consisted of a reciprocating water motor similar to those used in water-powered washing machines. It was mounted over the neck of the bag in a vertical position with its piston rod projecting from the lower end.

In order to take samples the piston was stopped at the top of its stroke, the stirring line lowered into the bag and a meter block fastened to the quadraped. Water samples could then be taken from the center of the bag using standard Nansen or Van Dorn bottles. Light measurements were taken by lowering a light meter into the water and comparing the output with a "deck cell" placed on the buoy. "Light" and "dark" bottles for photosynthesis measurements could be fitted to the racks on the stirring assembly, as illustrated. With extinction values (base e) between about 0.3 and 1.0 it can be shown that the rate of photosynthesis in a bottle suspended 2 ft above the center point of the bag should approximate to the mean rate from a plant cell in random motion throughout the entire water mass.

Fouling of the outside of the equipment, with a consequent loss of light penetrating into the bag, was restricted mainly to the upper surfaces and on the nylon netting. The growth, mainly a hydroid in our experiments, could be kept in check by throwing coarse crystals of copper sulphate onto the netting every few days.

We have used the above equipment successfully at Departure Bay (near Nanaimo, B. C.) where it was anchored some 300 ft from the end of a pier in water having a depth of at least 11 m at low tide. Full details of this work will be published later. In our experiments the bag was filled with water (32,000 U. S. gal) taken from a plastic pipe from a depth of over 20 m and filtered through a commercial diatomaceous earth filtration unit which removed all plant and animal life. The same water-line as that used to power the stirring motor was used for transporting water to the bag. The container was "innoculated" with a few hundred gallons of surface water, filtered through a 300-μ nylon net to remove most zooplankton. No animal populations were found to develop in the ensuing weeks. The temperature of the water in the bag kept to within 1°C of the temperature of the surrounding sea and the assembly appeared to be "seaworthy" to a remarkable extent, although it clearly should be used only in relatively sheltered waters.

We have reported the construction of this equipment so as to point out its proven feasibility. The apparatus is a most valuable tool for the study of primary productivity and could have many other applications, such as a study of zooplankton growth and grazing rates. We would be pleased to provide more detailed information to anyone contemplating the construction of a similar apparatus.

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Reprinted from ANALYTICAL CHEMISTRY, Vol. 33, Page 286, February 1961
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Highly Accurate Continuous Recording Differential Refractometer

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▶ **A recording differential refractometer capable of 0.5% accuracy in measuring 1×10^{-4} Δn difference in the refractive index of flowing solutions is described. Full scale deflection is obtained for 1×10^{-4} Δn at 60% amplifier output with a noise level of $\pm 5 \times 10^{-7}$ Δn. The potential range is 0.05 Δn. The cell compartments hold only 50 μl. The apparatus is sturdily built, and well protected against vibration and shock. Devices used for noise and stability control are described and discussed. Performance data are given. The apparatus is well suited to the continuous analysis and monitoring of chromatographic elution, and should be particularly helpful in extending the range of practical application for this technique.**

ALTHOUGH GAS CHROMATOGRAPHY frequently is preferred to elution chromatography as a working tool, in many cases the latter is still the analytical and preparative method of choice. This is true, for example, when mixtures of heat-sensitive and/or high molecular weight compounds are involved.

The use of refractometry as a complementary technique to this method was initiated by Tiselius and Claesson in 1942 (*16*). It resulted in the development of various types of automatically-recording apparatus, most of which are based on differential refractometry (*1–17*). Elution chromatography is greatly assisted by these instruments which eliminate the time-consuming eluate analysis. Unfortunately, their application is restricted by a practical sensitivity limit which, in general, is determined by noise level and instability rather than by actual lack of sensitivity. Thus none of the apparatus proposed hitherto, as judged from published performance data, is able to measure a difference of refractive index in flowing liquids of one unit of the fourth decimal place with an accuracy better than 10%. Yet in many cases, the total change in refractivity at the peak concentration of bands appearing in chromatographic eluate does not exceed $3 \times 10^{-6}\Delta n$ for major components, and $5 \times 10^{-6}\Delta n$ for minor ones. This situation, which arises either from very low eluate concentration, or from the solute-solvent refractive index difference being small, will be met frequently under conditions favoring a good separation. An apparatus extending the range of usefulness of recording refractometry to such cases is, therefore, desirable.

The following example will show the influence of noise upon apparatus performance.

Consider, for instance, Figure 3 in a paper by Glenn *et al.* (*8*) where record *d* (50% *S*) shows spurious fluctuations from the ideal straight line equivalent to ±3.3 chart units. From the value of *D* given in Figure 4 of the same paper for the Δn equivalent of one chart unit, i.e., $3.4 \times 10^{-6}\Delta n$ at 50% *S*, the value of the noise level can be deduced: it is $\pm 3.3 \times 3.4 \times 10^{-6}\Delta n$ or $\pm 1.3 \times 10^{-5}\Delta n$.

In absolute value, this noise level will remain the same regardless of the refractivity difference being measured. On the measurement of $1 \times 10^{-4}\Delta n$ it will induce an uncertainty of ±13%. A signal corresponding to $1 \times 10^{-5}\Delta n$ would be completely indiscernible from noise.

Noise, as reflected by spurious deflections of the recorder pen is of various origins. That which is due to temperature differences between reference and unknown liquids can be partially overcome by the arrangement proposed by Glasser and Troy (*7*). However, any arrangement of the analyzing cell which will increase its volume will lead to increased loss of definition in the chromatogram from eluate remixing effect. It is, therefore, imperative that the cell volume be kept as low as possible for the analysis of eluate from small laboratory columns. The arrangement proposed by Glasser and Troy derives its effectiveness from the fact that one of the solutions—the reference liquid—is kept stationary in a prismatic cell surrounded by flowing unknown. It is not nearly so effective when the technique of gradient elution is used. In such case the continuous change in composition of the eluting solvent must be matched exactly in the reference solvent. The liquid in the reference compartment cannot, therefore, remain stationary.

The apparatus described below is well adapted to these techniques since both reference and unknown liquid cell compartments are of equal and very small volume (50 μl.). It also can be adapted to the analysis of much larger effluent output by the use of a simple sampling bypass. In spite of a very small cell aperture resulting from the reduced cell dimensions, this apparatus is capable of measuring $1 \times 10^{-4}\Delta n$ as a full-scale deflection. This was obtained by improving the early model proposed by Zaukelies and Frost (*17*): first, by using a more sensitive recorder and increasing illumination efficiency, and second, by increasing the distance between photocell cathodes and analyzing cell, and using a larger proportion of amplifier output. It was not necessary to use a complex split-beam compensating device to reduce noise level to $5 \times 10^{-7}\Delta n$. This was accomplished as follows.

NOISE CONTROL

Mechanical Noise. Displacement of parts induced by external vibrations or shock are ultimately reflected as noise component through changes in the position of the light beam. This is controlled by the sturdiness of the parts and their firm attachment to a solid heavy base, which rests on foam rubber pads. The light source, a Monla lamp, features a very thick and tightly coiled filament connected to short sturdy supports. All connections to the apparatus are sufficiently flexible to dampen any vibration that may be transmitted through them.

Optical Noise. At the same time variation in light intensity is controlled by decreasing to 5 volts the nominal 6 to 8 volts normally used with the lamp. Under such conditions the filament itself becomes a current-regulating device. In addition, lamp life is considerably prolonged.

Light intensity is controlled further by using as a source of current, a precision voltage stabilizer described below.

Thermal Noise. Spurious refractive index changes of thermal origin

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are prevented through the careful thermostatic control of analyzing cell temperature.

Of particular importance are displacements of optical parts, resulting from mechanical distortion of apparatus components, under the influence of temperature changes. Such displacements are considerably amplified and reflected by erratic deflections of the light spot on the cathodes. To a large extent, thermostatic control of the analyzing cell assembly minimizes these effects in so far as the assembly is concerned. Thermostatic control of the lamp house is, however, just as important. The use of a low expansion heat coefficient cast iron base, and the absence of any other rigid connection between the three main parts of the apparatus, are further measures of thermal noise control. Housing the apparatus in a Fiberglas-padded box was also very helpful, provided an escape existed for excess heat from the lamp to the outside of the box; or better, provided the lamp housing was closed by a thermostated metal lid.

Careful control of the thermostated water supply is necessary. Control to $\pm 1/500°$ C. is obtained in the following manner. A 12-gallon glass tank is equipped with a toluene-expansion type regulator. The toluene reservoir, a coiled glass tube 150 cm. in length and 12 mm. in diameter, is connected to a mercury reservoir extending to a capillary tube fitted with adjustable electrical contacts. This operates a sensitive electronic relay actuating a 100-watt immersion heater in series with a powerstat. Adjustable water cooling is also provided to correspond to somewhat more than the heat (35 watts) removed from the apparatus. Careful balance of cooling and heat impulses induces smooth temperature control of the thermostated water which is sent to the apparatus by a centrifugal pump through heavily lagged tubing 1 inch in diameter. The latter extends along the whole length of the apparatus housing in the form of a manifold from which water is supplied to the various parts through individual rubber connections. Return to the bath is also effected through a similar manifold, both manifolds contributing to the control of temperature within the housing box.

Electrical Noise. The use of high quality components in the circuits, the exclusive use of carefully filtered direct current, the excellent shielding of circuit components, the location of components in a dry atmosphere, the avoidance of the proximity of strong magnetic fields, and the filtering of the alternating current to the power supply, constitute the main measures of electrical noise control. Aside from the particular mounting of the photocell, excellent insulation along the circuit leading to the amplifier from the photocell is necessary. Surface leakage is further reduced by application of Dow–Corning silicone dielectric compound Number 4 on and around all parts involved in this circuit. Particular attention is paid to proper grounding by selecting the most efficient ground connection available.

The control measures described above have resulted in reducing the electrical noise well beyond the limit set for overall noise level, and have reduced the time required to reach equilibrium to less than 1 hour after both lamp and amplifier have been switched on.

Electronic valve 5814 A, sturdiest and most reliable member of the 12AU7 family, may have to be selected for this use. In the course of our work, one among several new tubes proved to be unsuitable although no fault could be detected by ordinary testing methods. Under the conditions of use, however, a satisfactory tube could be used almost indefinitely. Twin phototube 5584 proved to be more efficient than the parent 920. Under the present favorable conditions (only 0.035 lumen per sq. cm.) it should last a considerable time. After several years of almost daily use, the only components needing replacement have been the dry cells, which, depending upon their quality, should be renewed every 6 to 12 months.

Noise Induced by Flowing Liquids. It is necessary to protect liquids flowing to the analyzing cell against temperature changes. A difference of $1/1000°$ C. is enough to produce a change in refractive index of the order of $4 \times 10^{-7} \Delta n$ equivalent to the upper limit set for noise level. Thus, not only must the narrow, preferably metal, ducts leading the liquids to the cell be made to pass through a simple tubular jacket where thermostated water is circulated, but as little temperature difference as possible should exist at their point of origin because of the limitations of the system as a heat exchanger.

Impurities such as dust and lint are easily removed by filtration. Bubbles are avoided by using liquids degassed by boiling and subsequently cooled in stoppered containers, and also by ensuring perfect tightness of all connections.

Improved performance results from flow control, and the use of moderate flow rates.

DESCRIPTION OF APPARATUS

The apparatus (Figure 1) consists of lamp housing *A*, photocell compartment *B*, analyzing cell assembly *C*, and amplifier *H*. The latter is connected to a 10-mv. full-scale Brown recorder and a 5-volt direct current regulated power supply not shown. Elements *A*, *B*, *C*, and *H*, are firmly attached by screws to *D*, a thick slab of cast iron. The thermostated water circulation system described above completes the equipment.

Lamp Housing *A*. Inner housing 1, entirely surrounded by jacket 2, is 0.025 inch wider than bulb 3 (Leitz Universal Monla bulb) which is screwed tightly in threaded Teflon receptable 4. The latter is held in the collar of lamp 5 by three setscrews of which one, 6, is shown. Lamp holder 5 is fitting exactly in housing 1 and is held in position by a setscrew passing through base *D* as shown. The end of this screw fits in a vertical groove (not shown) in the side of lamp holder 5. Thus only vertical movement is allowed to the lamp holder when the setscrew is loosened.

Jacket 2 is connected to a source of thermostated water through inlet 7 and outlet 8.

Centered on the optical axis of the apparatus and passing through the jacket is a cylindrical opening in which collimator lens holder 9 fits snugly. Details of 9 are given in Figure 2.

Photocell Compartment *B*. Figure 1, center, shows a top view of *B* with top panel and photocell arrangement removed. Below is shown a vertical section through *B*.

Photocell compartment *B* consists of an airtight box, obtained by assembling several panels fastened by screws to fixed front bracket 10 and base plate 11. The front bracket is fitted with flanged window 12 sealed by plane glass 13. The $1/8$-inch thick base plate bears photocell carriage 15, a $1/8$-inch thick rectangular plate screwed to sliding undercarriage 16. One of the 45° slanted sides of 16 slides against the 45° undercut in the apex of bracket 10, the other 45° slanted side sliding against guide 17, which is screwed to 11 and is adjustable for optimum efficiency in sliding motion.

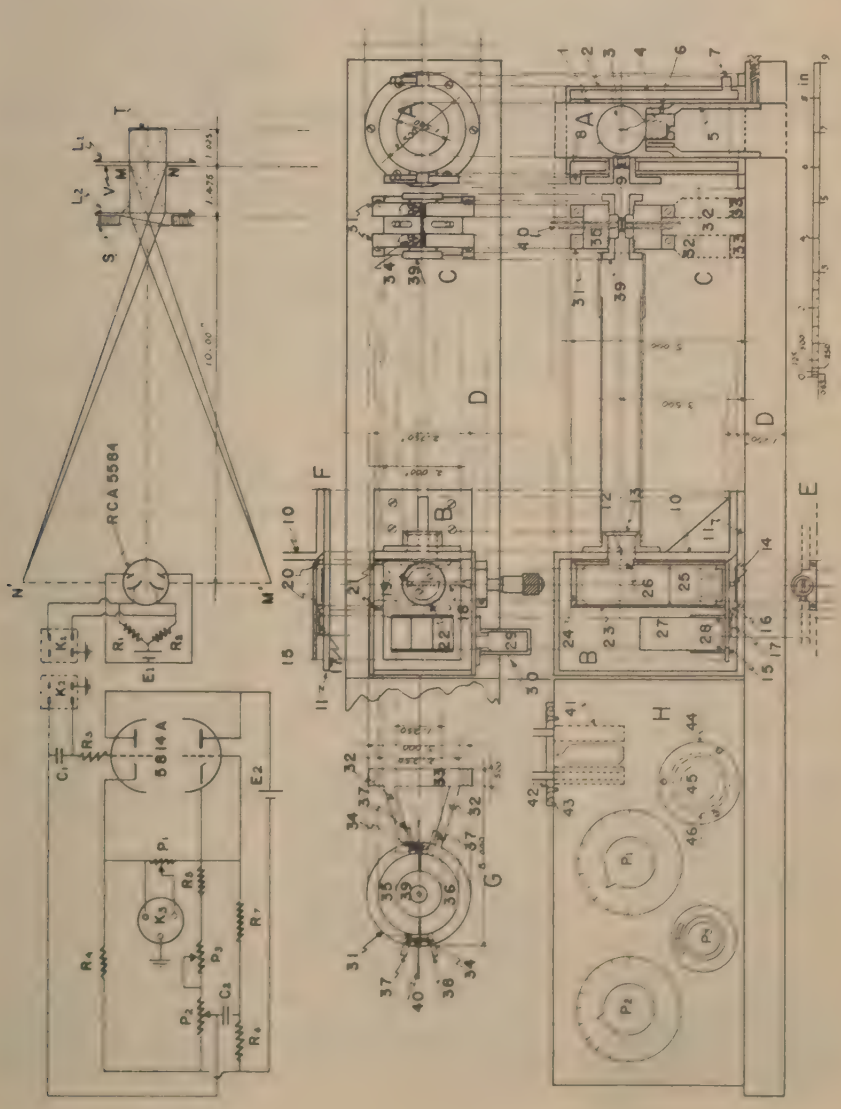
Part of a standard micrometer, $1/2$ to $1/16^2$ inch, is attached as shown in *E* to base plate 11. Micrometer rod 18, reduced to $1/8$ inch diameter, engages into $3/16$-inch diameter hole 14, drilled part way through the middle of undercarriage 15. A $3/16$-inch diameter steel ball 19 is lodged at the bottom of this hole to ensure smooth contact with the micrometer rod end. In *F*, showing a back view of the under carriage, are seen the openings of two holes 20 drilled part way through and on each side of the undercarriage. Each contains a tube 21, closed at one end. In both tubes is lodged a coiled spring pressing against the bottom of holes 20 and against the closed bottom of tubes 21 which are themselves leaning against the back panel of the compartment. A continuous pressure is thereby exerted upon the undercarriage, and backlash is eliminated.

In the middle of carriage 15 is screwed collar 22 over which photocell shield 23 fits tightly. The latter, a cylinder opened at the base and covered by lid 24, contains photocell 25. The cell is made to fit the shield tightly by winding a few turns of very thin Teflon tape

around the cell, above and below the electrode area. No socket is used, and the lead wires, which are soldered directly to the photocell prongs, are led out of the shield through three $^1/_4$-inch diameter Teflon disks wedged in holes drilled through the lower end of the shield. Rectangular opening 26, centered on the optical axis allows the light beam to reach the cathodes.

Three 30-volt hearing aid dry cells 27, part of the photocell circuit, are pinched between the sides of holder 28 which is screwed to the carriage. Completing the photocell compartment is drying agent holder 29, consisting of tube 30 with one open threaded end screwed over the threaded collar of a flange fastened to the top left corner of the panel. Tube 30 contains a gauze cartridge filled with silica gel.

Analyzing Cell Arrangement *C*. (See also front view *G*.) The cell block holder consists of two support-



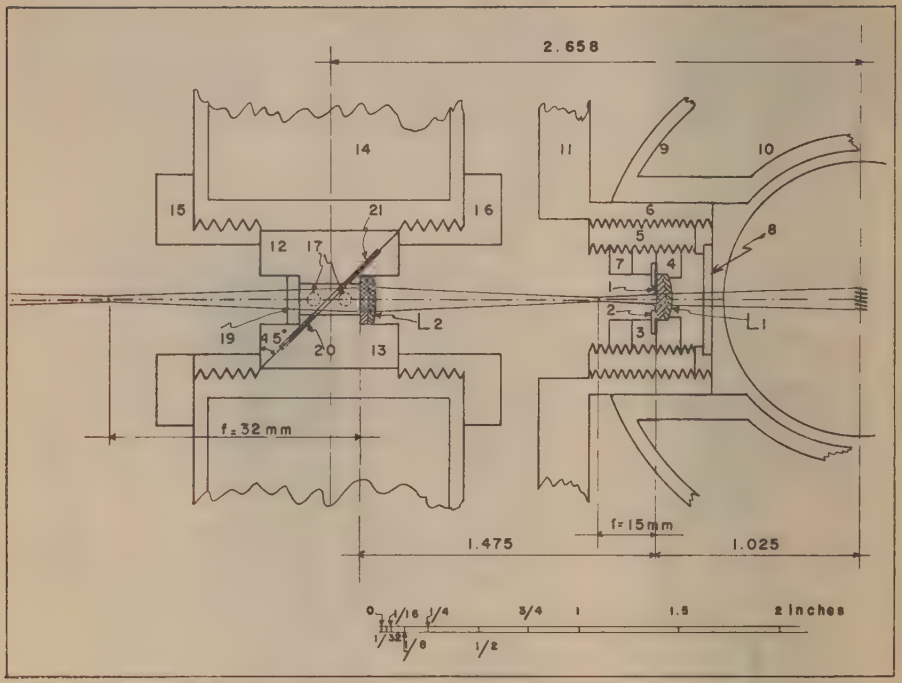


Figure 2. Horizontal section through lamp housing and analyzing cell arrangement showing details of optical system and cell

ing elements screwed to base *D*. Each consists of a strong ring 31, mounted on legs 32 welded to $^1/_2$-inch thick base bar 33. The rings are split by a vertical saw cut leaving a gap $^1/_{32}$ inch wide at the top and bottom. Tightening screws 34 brings the two halves of the rings together for a very firm hold on the thermostated cell block. The latter consists of two hollow halves 35 and 36, each of which is fitted with a water inlet 37 and outlet 38.

Tightening of rings 31 will bring the two halves of the cell block together, thus forming a cylinder the inside diameter of which is exactly that of the analyzing cell. The latter is positioned in the middle by two threaded knobs 39 fitting in the threaded ends of the cell block.

For further details see Figure 2.

◀

Figure 1. Diagram of apparatus

Top right, optical system
Top left, circuits of amplifier and photocell
Middle right, top view of apparatus
Middle left, front view of analyzing cell arrangement
Bottom right, vertical section through apparatus
Bottom left, amplifier

Amplifier *H*. (Also see circuit diagram top left of Figure 1.) The amplifier is housed in a completely airtight box immediately following the photocell compartment and connected to the latter by a Jones plug and receptacle (P-2404-SB, S-2404-SB) arrangement (on diagram K_1, K_2). The vacuum tube is surrounded by thermostated water jacket 41 fastened to the top panel of the amplifier by flange 42 fitted with O ring 43.

Drying agent holder 44 is screwed to the front panel by flange 45 fitted with O ring 46. The 1-inch diameter gauze tube (not shown), containing silica gel, fits over a collar on the inside face of the flange.

The amplifier circuit and photocell circuit parts are given below.

R1 = R2 = 20 megohm; R3 = 20 kiloohm; R4 = 50 kiloohm; R5 = 9 kiloohm; R6 = 400 kiloohm; R7 = 100 kiloohm. All are precision metal film Nobleloy resistors (Bayly Engineering, Ajax, Ontario) type NR-25, 1 W, tolerance 1%.

C1, 0.1 μf; C2, 0.01 μf., high quality condensers.

P1, P2, high quality rotary decade stud switch No. D-904-B/4 (Muirhead Instruments Ltd.).

P1, sensitivity control, 10 × 400 ohms; P2, coarse zero adjust, 10 × 500 ohms; P3, fine zero adjust., 1 kiloohm, 10-turn Helipot, A series fitted with counter.

K3, Amphenol 91-MC3M and 91-PC3F connected to recorder by Belden No. 8422 two-conductor microphone cable.

E1, 3 × 30-volt hearing aid cells.

E2, 90-volt dry cell.

R1, R2, and E, are housed in *B*.

Constant Voltage Power Supply. The current for the filaments of both amplifier tube and Monla lamp is 5-volt direct current obtained from a precision, ±0.01% regulation, voltage stabilizer. (No. 80710, Nylab, New York).

Optical System. A diagram of this system is shown at the top right of Figure 1. For clarity, the scale of the vertical dimensions has been made ten times that of the horizontal ones.

Filament T of the Monla lamp is approximately 2.5 mm. wide and 2 mm. high. Collimator lens L1, a plano-convex achromatic lens of 15-mm. focal length, projects a slightly enlarged image (3.4 mm. diagonal) of this filament on the plane face of plano-convex achromatic lens L2. The latter (focal length, 32 mm.) is also the front window of analyzing cell S (cell diameter 4

mm.). The diagram shows a geometrical construction of the filament image (dotted lines). On the other hand, lens L2 is focused on square diaphragm V (1.78 × 1.78 mm.) located against the plane face of lens L1. The diagram shows the construction of M^1N^1, the 18-mm. image of MN, which is the 2.5-mm. diagonal of diaphragm V. An image, (0.5 × 0.5 inch), of the diaphragm is being projected on the cathodes.

It is immediately apparent from the diagram that no light is intercepted by the cell. Diaphragm V is both the field stop and limiting aperture of the system. The arrangement is similar to the Köhler illumination used in photomicrography for the purpose of increasing illumination efficiency. At the same time it produces a sharp, square light spot on the cathodes, a desirable feature from the point of view of linearity of response, and results in a very compact arrangement of optical parts ahead of the analyzing cell.

Details of this arrangement are shown in Figure 2.

Diaphragm 1 is made of four straight edged strips of metal foil cemented one by one on a thin ($^1/_{32}$-inch) metal disk which has a $^1/_8$-inch hole in its center. The diaphragm is cemented on threaded mount 3. Cementing operations are conducted under a traveling microscope for accuracy in dimensions and centering. The diaphragm is brought in contact with plane face of lens L1 cemented in mount 4. Mount 5, holding the lens-diaphragm assembly, is held in position in lens holder 6 by threaded set ring 7. Heat absorbing glass disk 8 protects the assembly which furthermore, is surrounded by thermostated water jacket 9 of lamp house 10. Positioning of tightly fitting holder 6 is effected by manipulation of flange 11. Both this operation and thermal contact are helped by lubrication.

The analyzing cell consists of two truncated cylindrical halves, 12 and 13, identical in size. When pressed together in thermostated cell block 14 by threaded knobs 15 and 16, the two halves join by their 45°-slanted faces. Note that a vertical shallow groove cut shown in the middle of both top and bottom jaws of thermostated cell block 14 leaves space for $^1/_{16}$-diameter stainless steel solution inlet and outlet tubes 17 cemented in the cell walls.

Solution compartment 18 is fitted with cemented lens L2 while the solvent compartment is fitted with cemented 1-mm. thick plane glass 19.

Both compartments have been fitted with mica windows 20, 21. These are made of square sections of microscope cover glass cemented to the 45°-slanted faces in a 0.0300-inch deep square depression machined around the center. Care is taken in cementing all parts with epoxy resin cement curing at 90° C. to prevent excess cement from invading the planes of the cell chamber. In cementing 20 and 21 the cover glasses are brought over with the plane of the metal faces by pressing gently against a plane surface before curing. Mounting the assembly is accomplished by first heating the two half-cells, and all other parts such as cell blocks, knobs, and holders, in an oven at 110° C. Then a small drop of molten Canada balsam is placed in the center of one of the cover glasses, and the two half-cells are pressed together, avoiding any air bubbles between the glasses. All parts are then assembled while hot. The completed assembly is left to cool to room temperature before it is mounted on the apparatus base.

All inside surfaces except the cell walls, are painted with black optical finish.

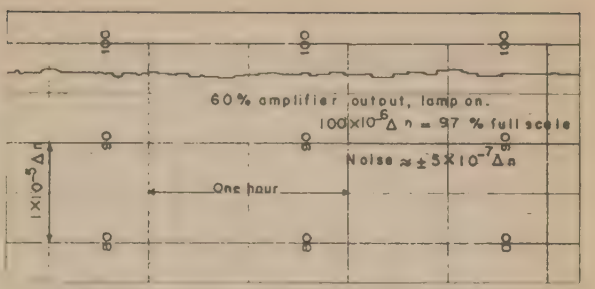


Figure 3. Record obtained at 60% output ($S = 6$), lamp on, showing extent of noise and total pen deflection for 1/1000-inch movement of photocell

PERFORMANCE DATA

From the simple theory of this type of instrument (*3*), the change in refractive index corresponding to $^1/_{1000}$-inch lateral movement of the photocell is expressed by

$$r = tg\,\alpha\,\frac{10^{-3}}{D}\,\Delta n \qquad (1)$$

where α is the boundary angle of the analyzing cell compartments and D the distance in inches between this boundary and the photocell cathodes along the optical axis.

In our case $\alpha = 45°$, $D = 10$. Therefore,

$$r = 1 \times 10^{-4}\,\Delta n \qquad (2)$$

A difference in refractive index of one unit of the fourth decimal place corresponds, therefore, to 1×10^{-3} inch displacement of the photocell carriage. Measurement of r was made by filling the cell compartments with aqueous ethyl alcohol solutions of known refractive indices determined with a precision Abbé refractometer. As an example, two solutions $A(n_D^{25} = 1.34518)$ and $B(n_D^{25} = 1.36104)$ differing by $\Delta n = 0.01584$ caused, when placed in the solvent and solution compartments, respectively, a deflection of the recorder pen corresponding to 0.156 inch. This was the distance the carriage had to be moved to bring back the recorder pen to a position obtained when both compartments contained solution *A*.

$$\text{Thus, } r = \frac{0.01584}{156} = 1.01 \times 10^{-4}\,\Delta n$$

Reversing the positions of the two solutions obtained a displacement in the other direction corresponding to 0.150 inch, indicating a nonlinearity of response over a $0.0300\Delta n$ range of only 2%.

Full advantage of the total range, which is about $5 \times 10^{-3}\Delta n$, could be taken by replacing the 400-ohm resistor in the first step of the sensitivity control by a 400-ohm Helipot potentiometer.

The number of chart units U_S corresponding to $1 \times 10^{-4}\Delta n$, or one 10^{-3} inch displacement of the photocell, constitutes an important value to be redetermined from time to time since it slowly decreases with aging of the dry cells. The most convenient way is to determine the number of chart units a corresponding to a 5×10^{-3} inch movement of the photocell when the sensitivity is set at 10% ($S = 1$). If S is the position number on sensitivity decade P1,

$$U_S = aS/5 \qquad (3)$$

Then,

$$F_S = \frac{100}{U_S}\,10^{-4}\,\Delta n \qquad (4)$$

represents the change in refractivity corresponding to full-scale deflection at a given sensitivity setting S.

With $a = 83$, and $S = 6$, $F_S = 1 \times 10^{-4}\Delta n$

The Δn value corresponding to any deflection of the recorder pen observed at sensitivity S is calculated from the corresponding number of chart divisions and the value F_S. The relative error on F_S is about 2% when obtained from a single value of a. With an average a value obtained through repeated determinations, the error is naturally decreased in proportion to the number of values involved.

Figure 3 shows the recording obtained at sensitivity position 6 (60% output)

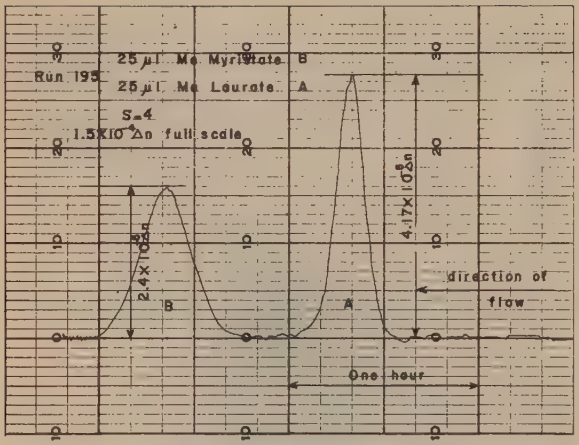


Figure 4. Chromatogram record

Obtained from reversed phase partition chromatography of mixture of methyl laurate and methyl myristate showing peak deflection corresponding to less than $5 \times 10^{-4}\Delta n$ for these major components

by moving the photocell 1×10^{-3} inch after adjusting the pen position to the zero mark, the same solvent flowing through both compartments of the cell. The noise level is reflected by deviations ($\pm 5 \times 10^{-7}\Delta n$) from a straight horizontal course indicative of high stability.

The micrometer was used in this instance to set approximately a deflection of $1 \times 10^{-4}\Delta n$. Its normal use is in calibrating the instrument. When the latter is used in analyzing effluent from a chromatographic column, recalibration is required once before each complete operation and only when the highest accuracy is desired.

A typical example of the use of the instrument in reversed phase chromatography is given in Figure 4 showing the chromatogram obtained at $S = 4$ from a 50% mixture (by volume) of methyl laurate and methyl myristate. Fifty microliters of this mixture were placed on a silicone-coated, 100- to 200-mesh crushed firebrick column moistened with iso-octane. Development with 82% (v./v.) iso-octane equilibrated aqueous ethyl alcohol at a rate of 300 ml. per hour resulted in complete separation.

When the solute involved is a liquid of known refractive index, the volume v_S of this liquid dissolved in a chromatographic band is given by

$$v_S = \frac{0.05\ AF_S f}{n_S - n_O} \qquad (5)$$

Where A is the area under the curve in square inches, F_S the quantity defined in Equation 4, f the rate of flow per hour, n_S the refractive index of the solute, and n_o, that of the solvent. Recorder chart speed: 2 inches per hour.

Applied to the first band in the chromatogram in Figure 4 (n_O = 1.36055; n_S = 1.4292; A = 1.13; $F_S = 1.5 \times 10^{-4}$; $f = 200 \times 10^3$ μl.), Equation 5 yields

v_S = 24.8 μl. (25 theoretical)

ACKNOWLEDGMENT

The authors are indebted to Russell E. Heffler, Physics Department, Dalhousie University, Halifax, Nova Scotia, Canada, for building important parts of the instrument.

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RECEIVED for review July 5, 1960. Accepted September 23, 1960.

PRINTED IN U. S. A.

Northern sea lions late in the season. At this time the harem structure breaks down, old bulls leave, and cows move with their pups to the water's edge.

THE NORTHERN SEA LION IN BRITISH COLUMBIA

PHOTOS COURTESY FISHERIES RESEARCH BOARD OF CANADA

by GORDON C. PIKE

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

THE sea lion is a paradox both by name and by reputation—a sea mammal variously called by the names of "sea wolf," "sea bear," and "sea lion," none of which are appropriate, and the least appropriate of which is "sea lion"; a creature of fame by reason of its great size and strength, majestic appearance and independent nature, but of infamy by reason of its depredations to fish and fishing gear; and imposter who, perhaps by way of apology, has submitted to the role of circus clown under the assumed name of seal."

The circus "seal" is usually a female California sea lion. The northern sea lion may be equal to the task in skill and intelligence but, because of its greater size and bellicose nature, is seldom trained and seldom seen in zoos.

The northern or Steller sea lion, *Eumetopias jubata*, is the only sea lion resident in British Columbia. Breeding colonies range widely from Herschel Island (69°N), through the eastern Bering Sea, along the Aleutian Islands, and southward along the west coast of North America to the California Channel Islands (33°N). In the western Pacific, it ranges southward from Kamchatka to Japan. The centre of distribution is Alaska with an estimated population of 150,000. British Columbia has an estimated population of 12,000; Washington—500; Oregon—1,000; and California—3,000. The total bulk of animal material represented

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FRB No. 646

by this population is enormous when one considers that a fully grown male measures 10 to 12 feet in length and weighs more than a ton, and a fully grown female measures 7 to 8 feet in length and weighs 600 to 800 pounds. The smaller California sea lion, *Zalophus californianus*, breeds farther south. Males of the California species sometimes visit the shores of southern British Columbia, where they mingle with herds of northern sea lions, but this species does not breed here.

Sea lions are included with fur seals in the family Otariidae because of certain anatomical resemblances to land mammals. Unlike the more aquatic "true seals" of the family Phocidae, the otariids have long hind flippers which bend forward at the ankle joint and permit considerable agility of movement on land. By reason of their small external ears, the family is sometimes referred to as the "eared seals." All seals, sea lions and the walrus are grouped in the Order Pinnipedia. Among land mammals the pinnipeds most closely resemble the dog-racoon-bear-weasel families of carnivores; but, despite these resemblances, the group merits independent rank and probably appeared long before there were any true wolves or bears. Ancestrally, the order is known to date back about 35 million years to the Miocene epoch.

A strange assortment of names has been borrowed from a variety of sources—birds, cattle, canines, and humans—to describe certain characteristics of seals and sea lions. Breeding localities are known as "rookeries"; breeding adults are "bulls" and "cows"; adults not yet breeding are "bachelors" and "virgins"; young of both sexes are "pups." Localities where sea lions come ashore for purposes other than breeding or pupping are called "hauling grounds." Family groups consisting of two or more cows in the care of a large bull are called "harems."

Most of the population of northern sea lions in British Columbia are concentrated on two major rookeries during the breeding season, which begins late in May and ends in August. These rookeries consist of several groups of inhospitable exposed rocks among the island chains lying to the west of Cape Scott and to the south of Cape St. James. A census taken by sea and air in the spring and summer of 1956-57 showed that 70 per cent of the total population and 90 per cent of the pups were located

A cow takes her newborn pup for a refreshing wash.

on these two rookeries. Smaller rookeries where few pups are born include North Danger Rocks in Hecate Strait and Sea Otter Rocks near the entrance to Rivers Inlet. Nearby rookeries in adjacent United States waters may modify the British Columbia population at certain times of the year. These include Forrester Island off Alaska (55°N) and the Jagged Islands off Washington (48°N).

Sea lions disperse widely throughout coastal waters and far out to sea at times other than the breeding season. The exact nature of this dispersion is not known, but evidence suggests a northern shift during winter months. The rookeries are inhabited during the breeding season almost exclusively by cows about to give birth, cows having recently given birth, newborn pups, and breeding bulls. Small groups of idle bulls may haul out on adjacent rocks. Hauling grounds, where only occasional pups are to be found, are located usually at great distances from the rookeries. Locations such as Joseph Rocks, Langara Rocks, Long Beach Rocks, and Solander Island are inhabited during the breeding season by bachelors, virgins, barren cows nursing yearlings, and some old bulls.

Life does get tedious, doesn't it? These 60-pound pups, one month old, wander or tumble aimlessly about on the rocks.

A small harem, consisting of a medium-sized bull and several cows with their pups.

A vessel approaching a sea lion colony during the breeding season is met by roars of defiance by bulls guarding harems and cows guarding pups. Many are reluctant to leave even when a landing is made or a shot is fired into their midst. At times other than the breeding season, any slight disturbance such as the awareness of an observer will result in a mass exodus from the rocks; from a distance the entire colony, looking like a brownish-yellow carpet, appears to slide towards the sea. At such times, the timid bleating of the abandoned pups mingles with the plaintive growls of the mothers and the defiant roars of the bulls, creating a continuous din. Undisturbed, the colony is usually fairly peaceful as the animals bask in the sunshine, sleeping or gently swaying to and fro. Pups crawl or tumble aimlessly and lazily about. Brief localized flurries of activity may result when a bull challenges an intruder or a cow attempts to relocate herself or is called upon to defend the ownership of a pup.

Groups of cows bearing pups begin to come ashore on the rookeries in May. These are followed by large bulls who take up stations among the cows and attempt to acquire harems. These harems of up to 20 or 30 cows are rather loose assemblages which continuously change in size and composition as cows promiscuously come and go, or as intruding bulls challenge the attendant master or otherwise disturb the status quo. Vocal threats or feigned attacks usually suffice to drive off an intruder, but sometimes fierce combat occurs between two males, and one, usually the intruder, will retreat bearing deep wounds about the head, neck or flippers. Cows frequently indulge in desultory squabbles, especially at times when there is dispute as to the ownership of a pup. On such occasions, the bull may intervene and restore domestic felicity.

The pups are sleek, black, sleepy little fellows weighing from 40 to

45 pounds at birth. They begin to appear the last week in May. Each cow bears a single pup until about the second week in July when pupping is finished. The cow cares for her pup tenderly and guards it jealously. She appears to reject all but her own offspring. During frustrated attempts to locate her own pup, which she recognizes mainly by smell, a cow may snatch another's and toss it roughly aside, sometimes 20 feet or more through the air. On occasion, two cows asserting ownership of a single pup will indulge in a tug-of-war with the prize which can do no more than helplessly bleat its disapproval. After giving birth, a cow will indulge in the luxury of a short nap or a refreshing swim. Left alone, the pups will eventually gather together in depressions or crevices in the rocks.

Evidence of the mothers' ability to relocate their offspring is shown by the rapidity with which the pups gain in weight and strength. Within two months of birth most pups weigh 70 to 80 pounds, and having learned in this hard school that might is right, will not hesitate to growl or snap at the source of any interference. By this time, most have been introduced to the water. Some have tumbled or been pushed in; some have been thrown in by irate cows; some have been carried or gently tossed in by their mothers. Pups can swim only feebly at birth, and many, lacking the assistance of their mothers in getting back on the rocks, must surely drown.

No elaborate courtship is displayed. Bulls unceremoniously check for cows in heat. This occurs a few days after birth and each cow is in turn serviced by the bull. As the season advances, the entire colony moves closer to the water's edge where cows, bulls and pups alike indulge their fancies or responsibilities in the water. Most of the large bulls, after having dutifully maintained their stations on the rocks for two months or more with little respite, gradually depart during the month of August and are replaced by an influx of younger bulls eager to exploit their opportunities.

Nursing continues for several months, but it is not known when the pup begins to obtain food from the sea. Cows bearing pups on the rookeries have abandoned their yearling offspring of the previous year. Barren cows found on hauling grounds during the breeding season are sometimes accompanied by nursing yearlings. Growth during the first year is rapid. Yearlings weigh from 150 to 200 pounds at the time of their first birthday.

A young bull northern sea lion, weighing about 1,500 pounds.

Mortality among the pups must be extremely heavy. On the rocks, some pups are trampled or otherwise abused by adults; some are drowned in pools or trapped in crevices in the rocks. The toll taken by disease appears to be slight, although this mortality factor has not been carefully investigated for the sea lion. Many are lost by drowning when storms wash them from the rocks. Some may succumb to exposure. Predators include sharks and killer whales, which take older animals as well as pups.

Food intake of northern sea lions is at a minimum at the height of the breeding season in June when bulls are preoccupied with maintaining harems and cows with pupping, caring for their newborn and breeding. Most of the 250 stomachs of adult males and females taken in British Columbia at this time of year have been empty. More food is found in the stomachs of young animals of both sexes and cows nursing yearlings on the hauling grounds. Stomachs with food are found to contain mostly octopus; also whiting, rockfish, dogfish, shellfish, salmon, herring, hake, lamprey and "scrap-fish." Some contained rocks, the exact purpose of which is not known.

At times other than the breeding season, sea lions follow runs of schooling fish such as herring, eulachon and salmon into the inlets. The appearance of sea lions coincident with fish runs does not

A small harem registers its vocal protest to the photographer. A closer approach might incite the bull to attack.

always prove the type of food on which they are feeding. On one occasion sea lions taken during a salmon run up the Klamath River in California were found to be eating lampreys. On another occasion sea lions taken in Barkley Sound during a rich run of herring were found eating dogfish, rockfish, octopus, skate, and hake, in addition to herring, the major constituent.

There can be no doubt that large quantities of important food fishes are taken by the northern sea lion, and that extensive damage is done to gear in the north Pacific. Species and gear affected most are salmon and halibut. Quantitative assessments of the amount and type of fish destroyed are, however, still unsatisfactory and can probably never be entirely acceptable because of the wide range of this mammal, the variability in its diet, and the difficulties in obtaining stomach samples which will adequately represent food consumption according to location and time. Damage by sea lions in British Columbia has been minimized in the region of Rivers Inlet, a rich salmon fishing area, by continuous shooting of pups and adults on the Virgin and Pearl Rocks which command the entrance to the inlet. The killing of pups and adults over a large area has had little effect on the overall size of the British Columbia population according to census taken in 1913, 1916 and 1956-57.

Local control of sea lions in some areas where a valuable fishery is being harmed can be effective, but wide-spread control, according to our present knowledge, would be impractical and unjustified. Recent efforts to utilize the species commercially as a source of mink food and leather have met with some success. It is unlikely that the population will ever be decimated or exterminated either by control measures or by commercial hunting because of the inaccessibility of many of its rookeries and hauling grounds and the relatively poor reward offered for the commercial product.

Much remains to be learned about this interesting animal and systematic studies are underway in both British Columbia and Alaska to provide a factual basis for evaluation of predation and effective measures for control. Information is accumulating on total numbers, fluctuations in population size and structure, food habits, behaviour, and life history. This rational approach to the problem encourages confidence that existing areas of conflict between industry, conservation for use, and aesthetics can be harmonized to mutual advantage.

THE METABOLISM OF INOSITOL IN SALMON

III. THE BIOCHEMICAL REACTIONS OF 2-C^{14}-MYOINOSITOL IN COHO LIVER[1]

H. Tsuyuki and D. R. Idler

Abstract

The labelling of some of the intermediates of 2-C^{14}-myoinositol metabolism in acid extracts of coho salmon (*Oncorhynchus kisutch*) liver was investigated. Inositol monophosphate was found to be extremely heavily labelled. The specific activity of glucuronic acid was less than half that of the glucuronic acid moiety of uridine-5′-diphosphate glucuronic acid. Glucose, galactose, glycogen, glucose-1-phosphate, and glucose-6-phosphate contained very little radioactivity. The labelling data are discussed in relation to the known biochemical reactions of inositol in plants and animals.

Introduction

The distribution of radioactivities in the various tissues (1), and in the liver nucleotide fractions (2) of coho salmon following injection of 2-C^{14}-myoinositol, was previously reported. Of the liver nucleotides, the glucuronic acid moiety of UDPGA[2] was heavily labelled, while UDPH and UDPAH fractions showed little or no activity.

In the present communication the same acid-soluble liver extracts were explored further to determine the labelling pattern of some sugars, sugar phosphates, and other intermediates of inositol metabolism.

Methods

Injection of the Salmon[3] and the Preparation of Perchloric Acid Extracts

A 2340-g male coho was injected intraperitoneally with 3.98 mg (22 μc total activity) of 2-C^{14}-myoinositol and killed 7 hours later. An untreated 1860-g male coho was killed at approximately the same time and the livers, kidneys, guts, and hearts from the two fishes were combined as previously described (1). These samples were designated C^{14}-coho I.

In another experiment, a 4125-g male coho was injected intra-arterially with 3.65 mg of 2-C^{14}-myoinositol (20.3 μc total activity) in the dorsal aorta in three 1.0-ml doses at intervals of 2½ hours as described previously (1). Each dose was followed immediately by an intraperitoneal injection of 5.0 ml of

[1]Manuscript received December 27, 1960.
Contribution from the Fisheries Research Board of Canada, Chemistry Section of the Technological Station, Vancouver 8, B.C.

[2]The following abbreviations are used in this manuscript: UDPGA, uridine-5′-diphosphate glucuronic acid; UDPH, uridine-5′-diphosphate hexoses; UDPAH, uridine-5′-diphosphate acetylhexosamine; AMP, adenosine-5′-phosphate; CMP, cytidine-5′-phosphate; CDP-inositol, cytidine-5′ diphosphate inositol; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; GA-1-P, glucuronic acid-1-phosphate.

[3]We wish to thank Mr. Robert McLaren, Chief Biologist, Department of Fisheries, for making arrangements for us to obtain the salmon, which were captured immediately before their arrival on the Capilano River spawning grounds.

F.R.B. No. 647

sterile 50% aqueous glucose solution. Four and one-half hours after the final set of injections, the fish was killed and the various tissues separated and stored. Samples from this fish were designated C^{14}-coho II.

The perchloric acid extracts of the liver were prepared by a slight modification (3) of the method of Hurlbert *et al.* (4).

Paper Chromatography

Chromatographic grade of Whatman Nos. 1 and 3 sheets, washed successively with ammonia, acetic acid, and methanol, were used (5). The chromatograms were subjected to descending development in Shandon[4] "chromatank" measuring 20 in. long by 8 in. wide by 20 in. high, usually at 20° C but also at 0° C when necessary to minimize decomposition. The solvent systems employed were as follows: IAW, isobutyric acid:ammonium hydroxide:water, 66:1:33; IAWE, same as solvent IAW but containing 10% ethanol; BPW, *n*-butanol: pyridine:water, 6:4:1; BAW, *n*-butanol:acetic acid:water, 4:1:5; aq. A, aqueous ammonia, pH 10.0; aq. B, water-saturated *n*-butanol; aq. BA, water-saturated *n*-butanol, containing ammonia in the bottom of the chromatographic tank.

When samples were eluted from chromatograms developed in IAW and IAWE, the ammonium ion was removed by treatment with Amberlite IR-120(H)[5] before reapplication to another chromatogram.

Location of Samples on Chromatograms

Ultraviolet-absorbing samples were located on a C-8016 Gordon Misco Ultraviolet Irradiator Blueprint[6] adapted to permit light from a clear G.E. germicidal lamp of maximum intensity at 253.7 mμ to pass through a Corning No. 9863 filter.[7] The hexoses were located by spraying with ammoniacal silver nitrate, and inositol, glucuronic acid, glucuronolactone as well as any other compound containing a vicinal glycol group by the periodate oxidation method of Yoda (6), except that the periodate concentration was reduced to 0.2% to increase the oxidation differential between the sample and the paper. Inositol could be detected at a level of 1 to 2 μg over an area of 2-cm diameter on Whatman No. 1 paper. Sugar phosphates and inositol phosphate were detected by the method of Wade and Morgan (7). To facilitate detection, the spots were viewed under ultraviolet light.

Analytical Methods

Hexoses were determined by the Folin and Malmros (8) method, glucuronic acid by a modification of the orcinol method of Meijbaum (9), and inositol by a slight modification (10) of the yeast assay method using *Saccharomyces carlsbergensis*.[8] When analyses were performed on samples eluted from paper

[4]Manufactured by Shandon Scientific Co. Ltd., 6 Cromwell Place, London, S.W. 7, England.
[5]Product of Rohm and Haas Co., Washington Square, Philadelphia 5, Penn., U.S.A.
[6]Manufactured by Microchemical Specialties Co., 1825 Eastshore Highway, Berkeley 10, Calif., U.S.A.
[7]This apparatus was modified by Mr. A. P. Ronald of this laboratory.
[8]The microbiological assay was carried out by Miss V. M. Chang of this laboratory.

chromatograms, a clear adjacent section of the chromatogram of equal size was eluted and an appropriate aliquot was used as a blank.

Results

Fraction A_3 for C^{14}-Coho II

This fraction, when chromatographed on Whatman No. 3 paper in solvent IAWE at 0° C, separated into four ultraviolet-absorbing bands. The largest and fastest-moving band corresponded to AMP in solvent systems IAW and IAWE and its base after formic acid hydrolysis was identified as adenine in solvents aq. A and aq. BA. The ultraviolet band moving just behind AMP moved like CMP in solvent IAW and after hydrolysis in formic acid the base was identified as cytosine in solvents aq. A and aq. BA. The remaining two faint and slower-moving ultraviolet bands were not investigated any further.

The radioactivity of fraction A_3 was concentrated in two areas of a chromatogram developed in solvent IAW. The major radioactivity band corresponded to glucuronic acid (4010 c.p.m.) and the minor band to glucuronolactone (196 c.p.m.). These two compounds accounted for virtually all the radioactivity in this fraction. Upon rechromatography in the same solvent system some of the glucuronic acid lactonized. The specific activity of the glucuronic acid determined by radioactivity measurements and by quantitative assay of the uronic acid on aliquots of the same sample was 1070 c.p.m. per μmole. There were 3.9 μmoles of glucuronic acid in this fraction.

Water Wash from Dowex-1-Formate Column

The first water-wash fraction referred to in the previous paper (2) and containing virtually all the radioactivity was freed of glycogen by the method of Stetten (11). The supernatant was concentrated and the residual glycogen precipitated. The combined supernatants from these operations were concentrated, dissolved in water, and deionized by passing through a mixed bed column of Amberlite IR-120(H) and IR-45 (OH). The eluant contained essentially all the radioactivity of the water wash indicating that little or no radioactivity was present in the glycogen.

One-quarter of the sample (2.5 ml) of deionized glycogen-free water wash was applied as a 7-in. streak across Whatman No. 3 paper and developed in solvent BAW for 40 hours along with glucose, galactose, and inositol as reference standards. The chromatogram was cut into strips horizontally along a line drawn across the reference standards spotted on either side of the unknown streak, it was eluted, and aliquots were counted. Any irregularities in the solvent front which occurred during a long-period chromatography would be detected in this manner. All of the radioactivity applied on the paper was recovered. The radioactivities were concentrated chiefly in the hexoses (135 c.p.m.) and the inositol (1238 c.p.m.) areas. Rechromatography of the hexose band in solvent BPW for 48 hours showed the presence of large amounts of glucose and a little galactose when sprayed with ammoniacal silver nitrate.

When another 2.5 ml of sample was treated in the same manner and the

hexoses eluted, 91 c.p.m. was present in the glucose band and 44 c.p.m. in the galactose band. The hexoses were determined quantitatively on an aliquot of the same sample used for radioactivity measurements. Expressed in terms of the entire sample, 5 μmoles of galactose of 35 c.p.m. per μmole specific activity and 78 μmoles of glucose of 4.7 c.p.m. per μmole specific activity were found.

Fraction A_5 of C^{14}-Coho II

One-tenth of fraction A_5 representing 5570 c.p.m. was streaked across Whatman No. 3 paper and chromatographed for 4 days at 0° C in solvent IAWE. Total separation of reference standards G-1-P, G-6-P, and GA-1-P was achieved. Inositol monophosphate, which moved 3.6 cm behind G-1-P was not completely separated from this sugar phosphate even after 4 days.

Of the total radioactivity of this fraction, 91% was recovered in the inositol monophosphate band, which, upon hydrolysis with 22% hydrochloric acid (10) followed by chromatography in solvent IAW for 2 days at 20° C, resulted in 90% recovery of the radioactivity in the free inositol area. Microbiological assay of the inositol indicated that 30 μg was present in fraction A_5. The inositol value must be considered as approximate since the amount assayed was near the lower limit of the microbiological assay. Quantitative assay of inositol monophosphate by total phosphate determination was not possible since a complete chromatographic separation between G-6-P and inositol monophosphate could not be achieved. On the basis of inositol assay, however, the specific activity of inositol monophosphate was 60,500 c.p.m. per μmole. Glucose-6-phosphate, G-1-P, and a trace of GA-1-P were detected in A_5 by chromatography.

Discussion

If the data for the free inositol content of male sockeye (10, 12) corrected for body weights are used to estimate approximately the content of inositol in the coho of comparable maturity used for intra-arterial injection (1), a figure of 199 mg is obtained. However, if it is assumed that the 3.65 mg of intra-arterially injected 2-C^{14}-myoinositol is present primarily in the kidney, liver, and alimentary tract after the relatively short duration of the experiment, a dilution with 23.7 mg of non-radioactive inositol is expected. The radioactive inositol, specific activity 622,000 c.p.m. per μmole, would be diluted to 83,100 c.p.m. per μmole and this is of the order of magnitude (60,500 c.p.m. per μmole) found for inositol monophosphate.

The specific activities of some of the intermediates of the metabolism of 2-C^{14}-myoinositol in the liver of coho salmon have been summarized in Table I. The specific activity of inositol monophosphate was found to be manyfold higher than any of the other components measured. Since its total radioactivity is only slightly less than one-half of the total radioactivity (2) of the acid extract, considerable inositol must be utilized in this way. Free inositol monophosphate, first isolated by Hawthorne and Hübscher (13) and Hübscher

TABLE I

Summary of specific activities

Compound	Radioactivity per μmole
Inositol monophosphate	60,500
Glucuronic acid from UDPGA	2,370*
Glucuronic acid (free)	1,070
Galactose	35
Glucose	4.7

*Value obtained from previous publication (2).

and Hawthorne (14) from guinea pig liver, could originate from direct phosphorylation of inositol, although Moscatelli (15) and Paulus and Kennedy (16) were unable to demonstrate an inositol "kinase" by in vitro incubation of inositol with tissue homogenates in the presence of ATP. It could also arise from the decomposition of CDP-inositol during acid extraction although Redman and Hokin (17) found this nucleotide inositol to survive the trichloracetic acid extraction procedure during its isolation from duck pancreas. The hydrolysis of inositol phosphatides could be another source. However, it is most likely that inositol is directly phosphorylated in vivo because of the high specific activity of its phosphorylated ester, when compared to the probable dilution of injected inositol. The ester could then be converted to CDP-inositol through reactions somewhat similar to the conversion of the C-1-phosphates of sugars and uronic acids to their corresponding UDP derivatives. CDP-inositol could be utilized further by conversion to inositol phosphatides by a series of reactions of the nature proposed by Agranoff *et al.* (18) and Paulus and Kennedy (16).

The experiments of Charalampous *et al.* with an enzyme system of rat kidney capable of cleaving the inositol ring point to glucuronic acid as being one of the first products of cleavage (9, 19, 20, 21). These authors were unable to find any intermediates in the conversion of inositol to glucuronic acid (21). In fish liver, however, the specific activity of the glucuronic acid moiety of UDPGA is more than twice that of free glucuronic acid. Although the specific activity data alone do not necessarily prove that UDPGA is a precursor of glucuronic acid in this conversion, they suggest that the eventual conversion of inositol to glucuronic acid may be accomplished either by a direct ring cleavage or possibly through a series of reactions leading first to UDPGA followed by its hydrolysis to the uronic acid. The manner in which such reactions occur is in the realm of speculation, since no data are yet available. The discovery of CDP-inositol (17) suggests that UDPGA could conceivably be formed from this source as well as from the presently known biochemical reactions outlined by Hassid *et al.* (22).

The low specific activities of the hexoses were not surprising since the hexoses are far removed from the chain of reactions involved in the eventual conversion of inositol to the hexoses. The very low labelling of glycogen is in agreement

with previous investigations where no measurable radioactivity was found in the liver glycogen isolated and purified from the water wash from Dowex-1-formate column of the acid extract of C^{14}-coho I.

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MICROECOLOGICAL FACTORS IN OYSTER EPIZOOTICS[1]

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Abstract

On oyster beds as elsewhere, mud-water interfaces exhibit a biological film harboring saprobic microorganisms. The abundance of these is directly proportional to the amount of decomposing organic matter present. Different communities of protozoa and bacteria characterize each of the levels of organic pollution, which may be defined biologically in terms of a modification of the "Saprobiensystem" of Kolkwitz and Marsson. While the microorganisms concerned are not conspicuous on clean substrata except where especially favorable conditions are afforded certain of them by some dead animal or plant, their general abundance is indicative of pollution. This follows overaccumulation of reducible deposits, initiated by irregular tidal flushing, freshets, and winter icing, and rendering the microenvironment anaerobic. *Ostrea edulis* and *Crassostrea virginica* survive temporary exposure to the direct effects of such conditions as well as to near-lethal temperatures, but, weakened thereby, become vulnerable to invasion by saprobes from the enriched biological film. Unless environmental conditions improve before the oysters lose ability to recover, abnormal mortalities will be hastened by the activities of these microorganisms, notably *Hexamita inflata* (Protozoa). Relevant literature is reviewed, and possible local remedies are discussed. Canadian data are supported by original observations from Wales and Pakistan. Among other new host and locality records, *Cristispira balbianii* (Spirochaetales) and cercariae of a bucephalid trematode are listed from *Ostrea belcheri* from Karachi.

Introduction

Mass mortalities of American oysters, *Crassostrea virginica* (Gmelin), occurred on Prince Edward Island in 1915. Since then, "Malpeque disease" (named for the bay where an outbreak was first noticed) has been widely reported from the island (100) as well as from New Brunswick and Nova Scotia (76, 77). The decline in the oyster fishery of the Maritime Provinces has been attributed to this malady, suggested explanations for which rival in number those who have investigated it. Whatever their cause or causes, the gross symptoms of "Malpeque disease" are gaping, thinness, poor shell growth, failure to spawn, and the development of yellow-green pustules (76, 100). Quite commonly, the victim fails to exhibit one or even more of these manifestations.

There appears to be a high degree of contagiousness with the death of up to 99% of the stock, and it has been claimed that resistant strains develop from survivors. Furthermore, suspect seed oysters were brought into Malpeque Bay from the United States a year before the first outbreak (76). These facts might perhaps be accepted as circumstantial evidence for viral causation, which has yet to be disproved. Logie (76) isolated at least two species of bacteria from the blood of mainland *C. virginica*, including some which became sick after transfer to Prince Edward Island. In this connection,

[1]Manuscript received December 5, 1960.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., with financial assistance from the National Research Council of Canada and the Fisheries Research Board of Canada.
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lesions resembling Needler's pustules had already been reported from Australian oysters (127), and had been thought due to bacteria. Needler (99) concluded that a slime mold (Mycetozoa) was probably responsible, and Richardson (124), the first to record flagellates (*Hexamita* sp.) and ciliates (*Orchitophrya* sp.) from Canadian oysters, also argued for protozoan causation. Interest in hexamitiasis was revived in 1950–51, when the condition was recognized in Holland and the Gulf of Mexico (89). The possibility that this is a specific and highly fatal disease of oysters is currently under investigation at Malpeque Bay (29).

A trial introduction of European oysters (*Ostrea edulis* L.) from Conway, North Wales, to St. Andrews, New Brunswick, was made in the spring of 1957. The consignment was regarded as disease-free. Before replanting, the animals were carefully freed from macroscopic epibionts to avoid accidental pest establishments (78, 95). A month after their arrival they had suffered a 95% mortality, attributed to prolonged air exposure in transit (95). During June, 1958, a visit to the St. Andrews Biological Station of the Fisheries Research Board of Canada provided opportunities for studying survivors of this batch. They proved to be in good condition, and all but seven, the gills of which had become infested with a ciliate normally commensal on clams (p. 455), were free from parasites. However, a second consignment had been imported from the same source by air freight in the spring of 1958. Many of these had become thin and watery, and their examination disclosed ciliates on the mantle and (in one instance) *Hexamita* in the intestine. Several diseased American oysters sent down from the substation at Ellerslie, Malpeque Bay, also yielded protozoa, including *Hexamita*.

The European oysters studied had all been held in Sam Orr Pond, a brackish inlet of Passamaquoddy Bay some 60,000 square meters in area located near St. Andrews. Three hundred and fifty of the 1958 batch were now transferred to Ellerslie, some being held there in the brackish waters of the Bideford River and others in laboratory tanks. By late February, 1959, all were dead, about two-thirds of those examined harboring *Hexamita* (29). On March 5, 1959, a tray with part of the 1958 consignment was brought up from beneath 0.7 m of ice at Sam Orr Pond, and 98% of the oysters proved to be gaping or dead. Seven were sent to the Institute of Parasitology, but failed to arrive until the afternoon of the 12th. Although decomposing by then, all had huge numbers of active trophozoites of *Hexamita* throughout the tissues and over the body surfaces. The flagellate was duly recorded from all oysters remaining in the tray in question (28, 95).

As it strongly resembled *Hexamita inflata* (p. 469), a well known polysaprobe (74) occurring in polluted brackish (19) as well as fresh waters and long ago recorded from oysters and oyster beds in France (16), mud samples were requested. These were obtained from Sam Orr Pond from beneath 0.65 m of ice on April 7, and proved to support a number of polysaprobic flagellates including undoubted *H. inflata*. Two weeks later, when the last of the ice had melted away, it was found that there were no survivors of either importation. On the other hand, approximately 60% of 200 oysters of the 1958 shipment, held in the laboratory at St. Andrews, were still alive at the end of April (28).

A third consignment from Conway was air-freighted to St. Andrews, via Montreal, in 1959. These oysters arrived on May 2, and 11 of them, after spending a week end in salt-water tanks at the Biological Station, were received at the Institute of Parasitology on May 6. All were still alive, and although two bore a few colorless flagellates (*Monas guttula* and *Rhynchomonas nasuta*) on the gills, none harbored any *Hexamita* whatsoever. At the same time, seven survivors of the 1958 shipment, overwintered in the Biological Station laboratory, were received for study. Three were at an advanced stage of decomposition on arrival, and yielded only bacteria. The other four (in one of which ciliated epithelium was no longer active) harbored *H. inflata*, trophozoites being present on the body surfaces and abundant in the digestive gland and blood.

The oysters imported in 1959 were now planted, most of them in Sam Orr Pond. Some were subsequently transferred to Ellerslie while the remainder were established at the Biological Station (both in tanks and at the wharf, below low-tide mark) and in Oak Bay, a few miles west of St. Andrews. Oak Bay was the site of an abortive plant of American oysters in 1896, from which the establishment of *Asterias forbesi* in the Bay of Fundy is said to date (44). Intermittent examinations made by Mr. P. J. Downer up to July 16 revealed no flagellates in either healthy or moribund oysters at Sam Orr Pond. On July 24, however, 25 gapers all proved to be heavily infected with *Hexamita* (28).

A second period was spent at the St. Andrews Biological Station throughout August, 1959. On July 31, only 5 of 13 gapers from Sam Orr Pond had been found positive for *H. inflata*. The apparent decrease in incidence continued, for on August 6 six of eight gapers proved negative, as did four oysters in good condition on the 10th, and two decomposing examples on the 12th. Summer passed without any records of hexamitiasis from Oak Bay or from the oysters kept submerged at the Biological Station wharf. As summer progressed, though, the survivors of the 1958 batch overwintered at the laboratory continued to die, all literally teeming with *H. inflata*. By August 31, only 49 (25% of those placed there early in the preceding winter) remained alive (28).

It had been concluded (28, 95) that the mortalities of 1958–59 among introduced European oysters resulted from prolonged exposure to the low water temperatures of a severe winter. At the same time, it had become abundantly clear that neither the initial failure to establish *O. edulis* in New Brunswick nor epizootics among American oysters could be evaluated properly without clearer appreciation of the role of *Hexamita*. The microbiology of European oysters and their immediate environment at Sam Orr Pond thus received considerable attention during the August, 1959, visit to the Biological Station. Brief visits to Conway, North Wales, and to Karachi, Pakistan, made possible by a World Health Organization assignment on another matter in the following month, and to Ellerslie, Prince Edward Island, in July, 1960, provided additional pertinent information. The data are summarized and analyzed herein, morphological accounts of all the microorganisms except *H. inflata* (p. 469) being held over for further papers.

Oysters and Other Organisms

Shore invertebrates sedentary for most part of their life are forced into intimate relationships with an astonishing variety of plants and animals. Dollfus (26) lists organisms competing for food with oysters to the disadvantage of the latter, or smothering them, either directly, as in the case of algae of the genera *Cladophora*, *Ulva*, and *Zostera* (125), and the gastropod *Crepidula fornicata* (78), or by causing (as does the common edible mussel, *Mytilus edulis*) the rapid accumulation of silt. Perhaps the oddest relationship between these molluscs and plants concerns the balloon weeds, *Colpomenia* spp., phaeophyceous algae having a vesicular thallus that becomes distended with gas. Thick masses of *Colpomenia* growing upon oysters may drag them from their anchorage to the surface, where winds and currents bear them away (84, 126).

In the three centuries since Auzot (5) drew attention to polychaete infestations of European oysters, a great deal has been written concerning predators, commensals, and parasites of these molluscs. At all stages of their life, enemies prey upon them. Prodigious numbers of their eggs are destroyed by copepods and other plankton feeders. Many young oysters fall victim to crabs, which, on occasion, extract even adults from their shells. A full list of predators would include octopi, fish, and wading birds (26). There is general agreement, though, that starfish of several genera and species are the most widespread and destructive of the animals opening and eating oysters. Polyclad turbellarians stand close to the border line between predation and parasitism in their habits, and sometimes attain local importance (63, 1 6). Notable among these is *Stylochus inimicus*, often referred to as the "oyster leech", a misnomer that should be abandoned in view of the occurrence of a true leech, *Ostreobdella kakibir*, on *Crassostrea gigas* in Japan (112). Predation is most evident below the low-tide mark (147).

As might be expected, many organisms live as epibionts upon, or bore into, the shells of oysters. These range from filamentous bacteria, boring fungi (*Ostracoblabe implexa*), and various perforating algae (e.g., *Ostreobium quecketti*) (26), to representatives of almost all marine animal phyla: Verrill (139) deals with numerous North American examples, and the most exhaustive study of the *O. edulis* shell fauna to date (69) includes detailed records of Protozoa (Foraminifera, folliculinid ciliates), Porifera (Calcarea, Monaxonida, Cornacuspongida), Coelenterata (Hydrozoa, Scyphozoa, Anthozoa), Platyhelminthes (rhabdocoele turbellarians), Nemertea, Nematoda, Annelida (Polychaeta, Oligochaeta), Arthropoda (Crustacea, Arachnoidea), Mollusca (Loricata, Gastropoda, Lamellibranchiata), Prosopygia (Bryozoa), Echinodermata, and Chordata (Tunicata). Only two members of this formidable array (the boring sponge, *Clione celata*, and the polychaete, *Polydora ciliata*) burrow deeply enough or increase in numbers sufficiently to endanger the host in the Dutch waters where Korringa's studies were conducted, the shell fauna being regarded as primarily adventitious (69). Such organisms are nevertheless always liable to flourish to the oyster's detriment through abnormal biotic disturbances. Thus Kavanagh (64) found Japanese *C. gigas* imported to Louisiana much more adversely affected by *Polydora* than were local American oysters. Again, *Polydora* is liable to destruction through undue

dilution of sea water following freshets. Gross putrefaction within shell blisters caused by the worms may then lead to the host's death (149). The Australian device of raising oyster trays off the bottom to minimize polychaete attack has an opposite effect on the Connecticut coast (79). There, however, the worm is not responsible for serious mortalities, although the numerous shell blisters resulting from heavy infestations render oysters unsightly and less saleable (85).

Polydora hoplura and allied species are probably more injurious than *P. ciliata* through their habit of dwelling within the shell valves (69). Also located here are various commensals from microorganisms to higher forms such as the hydroid, *Eugymnanthea ostrearum* (94), and the parasitic crab, *Pinnotheres ostreum* (136). In New Jersey the naked goby, *Gobiosoma bosci*, commonly shelters within empty lamellibranch shells, and there is a report of a 21-mm example of this fish from a 7-cm American oyster, in the right dorsal body wall of which it had excavated a bay where it had lived for at least a year (105).

In lighter vein, before turning to internal commensals and parasites, there are records not only of oysters living as epibionts themselves, upon the carapace of crabs (75), but also of a redoubtable example left overnight on a pantry floor and reputed to have killed mice by closing its shell valves upon them (91)!

Viruses have not been isolated from shellfish, although these harbor a variety of bacteria. Much of the relevant literature concerns the normal intestinal flora (9), or species which happen to be thriving in the surrounding medium (10, 38, 46, 92). Decomposing oysters naturally teem with bacteria (59), and the genus *Serratia* has been indicted as a cause of pinkness (98), a condition for which yeasts are also responsible (58). The alimentary tract harbors representatives of genera common in sea water charged with organic matter (*Spirillum*) and marine ooze (*Saprospira*) (11, 24), and well-known commensal spirochaetes (*Cristispira* spp.) associated with the crystalline style of healthy oysters (17, 24, 32, 110, 113).

No bacteria clearly incriminated as specific pathogens are known from this host. Giard (45) indeed described one (*Myotomus ostrearum*) from European oysters suffering from "Maladie du pied", but Dollfus (26) was not only sceptical as to the bacterial nature of this condition but held its status so doubtful as not to warrant further use of the name. *M. ostrearum* is not mentioned in the Sixth (revised) edition of Bergey's Manual of Determinative Bacteriology (11). Orton (113) quotes Cerruti for the information that during the great oyster mortalities at Taranto, Italy, in 1919-20 no pathogens were recognized among the many bacilli, spirilla, and cocci isolated from sickly specimens, although while the disaster was at its height, "there were on the intestine of the oysters and on the gills millions of a filiform bacterium very long and motionless. . . ." This is strongly suggestive of sewage bacteria, *Sphaerotilus natans* or *S. dichotomus*, and it should be noted that investigations during the 1920-21 mortalities on the British coast resulted in the culturing of the latter species, and a *Vibrio* too, from dying oysters (31, 114). The former organism, however, was not considered a primary cause of death, and as for the *Vibrio*, Orton (114) states that this was fed to sound oysters "which, it would appear, seem to rather enjoy it".

A specific fungal parasite, *Dermocystidium marinum*, occurs in American oysters on the South Atlantic and Gulf coasts of the U.S.A., causing considerable warm-weather mortality in waters of relatively high salinity (123). The gregarine protozoon, *Nematopsis ostrearum* (121), is widespread in the same area and host, but does not appear responsible for unusual mortalities (42, 73). Harmless amoebae (*Vahlkampfia patuxent*) have been recorded from the intestine of *C. virginica* (54), and several saprozoic and bacteria-feeding protozoa have been listed from oysters by authors (16, 17, 102, 113, 129) who did not view them as primarily injurious. As already stated, *Hexamita* has been ranked high among oyster pathogens (89, 124).

Bucephalus haimeanus and *B. cuculus* are trematode cercariae parasitizing European and American oysters respectively (55). Bucephalids have also been recorded from *Ostrea commercialis* and *O. angasi* in Australia (128). *B. cuculus* occurs in areas of low salinity, and has been reported from New Jersey to Texas. Heavy infections cause parasitic castration of the host, although this has been declared gastronomically beneficial, the oysters concerned remaining fat and of excellent flavor all summer (96). Menzel and Hopkins (96), in fact, record their personal preference for the taste of *Bucephalus* sporocysts over that of oysters! Other trematodes from these molluscs include *Proctoeces ostrea* from the genital gland of *C. gigas* (37), and three further species, together with three nematodes and the pearl-stimulating cestode (132, 133) from a distant relative, the pearl oyster (*Margaritifera vulgaris*) in Ceylon.

Common mussel copepods of the genus *Myticola* sometimes spread to *Ostrea edulis* in European waters (8) and to *O. lurida* and *C. gigas* on the west coast of North America (111), without appearing to do much harm to the new host. Oyster parasites reported from other animals in nature or transmitted to them in the laboratory include *Dermocystidium marinum*—for which the quahaug, *Mercenaria mercenaria*, and the soft-shell clam, *Mya arenaria*, serve, albeit poorly, as experimental hosts (123)—and *Bucephalus haimeanus*, which may also infect cockles (55). As might be expected, members of the shell fauna frequently settle on other sedentary animals.

Further information concerning the predators, epibionts, and endobionts of oysters may be had from Dollfus (26), Korringa (69), and the bibliographies of Baughman (6) and Ranson (122). Enough has been said to sketch the complexity of the interrelationships between oysters and other occupants of their biotope, and to serve as a background to the following sections.

Methods

Most of the organisms listed were first studied alive by phase contrast. Methylene blue was used for vital staining, and nigrosin for dry relief slides. Smears for iron haematoxylin staining were made on albuminized $\frac{3}{4}$ in. square cover slips (No. 1), and fixed in Davis' modification of Worcester's fluid (75 cc saturated solution mercuric chloride, 20 cc formaldehyde 40%, 5 cc glacial acetic acid). Sections were cut at 7 μ from Bouin-fixed material, and stained with haematoxylin/eosin. A phase contrast microscope was also used at Conway, where, as at Karachi a week later, bright field observations were made with a pocket-sized "McArthur" microscope having an attached battery-

powered illuminant. During these visits, cover slip smears made and fixed as above were stored in iodized alcohol in 2 in. by $\frac{1}{2}$ in. plastic tubes pending my return home.

Materials

Cristispira balbianii (Certes) was found in European oysters at St. Andrews and Conway, in American oysters at Ellerslie, and in *Ostrea belcheri* at Karachi. The only other widely reported specific associates of oysters discovered were bucephalid trematodes. *Bucephalus cuculus* McCrady was recorded from one (3%) of the 30 American oysters dissected, while unidentified bucephalid cercariae were found in three (15%) of the 20 *O. belcheri* examined at Karachi.

The Ellerslie oyster parasitized by *B. cuculus*, and a second example, had astomatous ciliates in the digestive gland. These were referable to *Orchitophrya*, a genus already recorded from Prince Edward Island oysters by Richardson (124), and were morphologically identical with *O. stellarum* Cépède. This species causes parasitic castration of starfish (15, 141), and was first reported from a North American host (*Asterias forbesi*, at Milford, Connecticut) in 1935 (3). Its incidence on Canadian Atlantic shores, where the cold-water purple star (*Asterias vulgaris*) predominates, is unknown.*

A second ciliate, the thigmotrich *Incistrocoma pelseneeri* Chatton and Lwoff, was present on the gills of seven (20%) of 35 European oysters of the 1957 importation, when these were taken from Sam Orr Pond in June, 1958. This commensal of soft-shell clams and several other lamellibranchs (70) has not been reported from oysters before.

It was not possible to identify free-living bacteria other than *Spirillum* and *Sphaerotilus*, as culture facilities were not available. Algal determinations were not made either. Other primarily free-living microorganisms recorded from oysters, their shells, and the surrounding mud are listed in Table I. Tables II and III show associations of such organisms, commensals, and parasites with healthy, gaping, and dead European and American oysters.

Discussion

Obligate and Facultative Parasites

Cristispira balbianii is a commensal, the absence, not the presence, of which is a sign of unhealthiness of the host. First described from European oysters (16, 17), and later from *C. virginica* (101, 110), it is now listed for the first time from *Ostrea belcheri* and Pakistan. It normally occurs in the crystalline style, which soon breaks down after the host is removed from the water or if it becomes sickly (113). The spirochaetes then spread throughout the gut contents and over the gills and mantle (113). Style reformation does not take place under anaerobic conditions (Berkeley, quoted by Nelson (104)), during which *Cristispira* occurs in very small numbers, if at all. In the present studies it was never found in gaping oysters lacking a style and under invasion by saprozoic organisms. Perhaps amylolytic ferments produced by a normal style (104, 106) preclude such invasion of the healthy animal?

*Since this went to press my attention has been drawn to a research note by G. F. M. Smith (Science, **84**, 157 (1936)) recording *O. stellarum* from about 25% of a small collection of female *A. vulgaris* from Malpeque Bay oyster beds.

TABLE I

Normally free-living organisms recorded from oysters and their immediate vicinity
(C = common, R = rare, X = present, incidence indicated in Tables II and III)

Microorganism (Relevant references follow each name in parentheses)	*O. edulis* St. Andrews, N.B. In mud nearby	*O. edulis* St. Andrews, N.B. On shell	*O. edulis* St. Andrews, N.B. In or on oyster	*O. edulis* Conway, N. Wales On shell	*O. edulis* Conway, N. Wales In or on oyster	*C. virginica* Ellerslie, P.E.I. On shell	*C. virginica* Ellerslie, P.E.I. In or on oyster	*O. belcheri* Karachi, Pakistan On shell	*O. belcheri* Karachi, Pakistan In or on oyster
Bacteria									
Eubacteriales									
Spirillum sp.	C	C	X	—	—	C	X	—	—
Chlamydobacteriales									
Sphaerotilus dichotomus (Cohn) (114)	C	C	X	—	—	—	—	—	—
Protozoa									
Mastigophora									
Euglenoidina									
Anisonema acinus Dujardin (19, 47, 71, 142, 148)	R	R	—	R	—	C	—	—	—
Astasia sp. (71)	R	R	—	—	—	—	—	—	—
Peranema trichophorum (Ehrenberg) (19, 71, 131)	—	—	—	R	—	—	—	—	—
Marsupiogaster picta Faria *et al.* (33, 148)	—	—	—	C	—	—	—	C	—
Tropidoscyphus octocostatus Stein (14, 33, 47, 71, 142)	—	—	—	R	—	—	—	—	—
Dinoflagellata									
Glenodinium foliaceum Stein (19)	R	—	X	—	—	—	—	—	—
Prorocentrum micans Ehrenberg (19, 23, 71, 131)	R	—	X	—	—	—	—	—	—
Protomonadina									
Bodo edax Klebs (19)	C	C	X	—	—	C	X	—	—
Bodo rostratus (Kent) (19, 65, 131, 142)	C	C	X	—	—	C	X	—	—
Monas guttula Ehrenberg (33, 47)	R	—	X	—	—	—	—	—	—
Monas minima Meyer (19)	C	C	X	—	—	C	X	—	—
Oikomonas termo (Ehrenberg) (19, 71, 140)	C	C	—	—	—	C	—	—	—
Rhynchomonas nasuta (Stokes) (33, 47, 90)	C	—	X	—	—	—	—	—	—
Polymastigina									
Dallingeria drysdali Kent (65, 71)	C	—	X	—	—	—	X	—	—
Hexamita inflata Dujardin (16, 17, 19)	R	R	X	—	—	R	X	—	—
Trepomonas agilis Dujardin (19, 71)	R	—	X	—	—	—	—	—	—
Sarcodina									
Amoebina									
Amoeba radiosa Ehrenberg (131, 148)	C	C	X	R	—	—	—	—	—

TABLE I (*Continued*)

Normally free-living organisms recorded from oysters and their immediate vicinity
(C = common, R = rare, X = present, incidence indicated in Tables II and III)

Microorganism (Relevant references follow each name in parentheses)	*O. edulis* St. Andrews, N.B. In mud nearby	*O. edulis* St. Andrews, N.B. On shell	*O. edulis* St. Andrews, N.B. In or on oyster	*O. edulis* Conway, N. Wales On shell	*O. edulis* Conway, N. Wales In or on oyster	*C. virginica* Ellerslie, P.E.I. On shell	*C. virginica* Ellerslie, P.E.I. In or on oyster	*O. belcheri* Karachi, Pakistan On shell	*O. belcheri* Karachi, Pakistan In or on oyster
Ciliata									
Holotricha									
Chilodonella cucullulus (O. F. Müller) (14, 71)	R	C	X	—	—	—	—	—	—
Cinetochilum margaritaceum Perty (62, 71)	—	—	X	—	—	R	—	—	—
Cohnilembus verminus (O. F. Müller) (14, 33, 62, 71, 143)	R	C	X	—	—	C	X	—	—
Colpidium campylum (Stokes) (62)	—	—	X	—	—	—	X	—	—
Colpidium colpoda (Ehrenberg) (14, 113, 143)	—	—	X	—	—	—	X	—	—
Cyclidium glaucoma O. F. Müller (33, 71, 74)	C	C	X	—	—	C	X	—	—
Enchelyodon sp. (16, 17)	—	C	X	—	—	C	X	—	—
Frontonia marina Fabre-Domergue (62, 143, 148)	—	—	—	—	—	R	—	—	—
Lacrymaria coronata Claparède and Lachmann (67, 148)	R	—	—	R	—	—	—	—	—
Lionotus fasciola (Ehrenberg) (14, 33, 62, 71, 74, 143, 148)	R	R	X	—	—	—	—	—	—
Paramecium sp. (71)	C	R	—	—	—	R	—	—	—
Platynematum sociale (Penard) (62)	—	—	—	—	—	—	X	—	—
Pleuronema marinum Dujardin (14, 71, 148)	C	C	X	—	—	C	X	—	—
Urocentrum turbo (O. F. Müller) (62, 71, 74)	—	—	—	—	—	R	X	—	—
Uronema marinum Dujardin (14, 71, 90, 143)	R	R	X	—	—	R	X	—	—
Uronema pluricaudatum Noland	R	R	—	—	—	—	—	—	—
Unidentified tetrahymenids (72, 113)	R	—	X	—	—	—	X	—	—
Spirotricha									
Aspidisca costata (Dujardin) (62, 71, 74)	R	R	X	—	—	C	X	—	—
Condylostoma patens (O. F. Müller) (14, 33, 62, 71, 148)	—	—	—	R	—	—	—	—	—
Euplotes charon (O. F. Müller) (1, 33, 62, 74, 114, 148)	R	C	X	R	—	R	X	C	—
Folliculina sp. (2, 69)	—	—	—	R	—	—	—	R	—
Holosticha kessleri (Wrzesniowski) (14, 62, 71, 148)	—	R	X	R	—	—	—	—	—
Keronopsis rubra (Ehrenberg) (62, 71, 130, 148)	R	—	—	—	—	—	—	R	—
Uronychia setigera Calkins (14, 71)	—	R	—	—	—	R	—	—	—
Uronychia transfuga (O. F. Müller) (33, 71, 143, 148)	—	—	—	—	—	R	X	—	—
Uronychia sp.	—	—	—	—	—	—	—	R	—
Urostyla grandis pallida Entz (62)	R	—	X	R	—	R	X	—	—
Peritricha									
Zoothamnium sp. (71)	—	—	—	—	—	R	—	—	—

TABLE I (*Concluded*)

Normally free-living organisms recorded from oysters and their immediate vicinity
(C = common, R = rare, X = present, incidence indicated in Tables II and III)

Microorganism (Relevant references follow each name in parentheses)	*O. edulis* St. Andrews, N.B. In mud nearby	*O. edulis* St. Andrews, N.B. On shell	*O. edulis* St. Andrews, N.B. In or on oyster	*O. edulis* Conway, N. Wales On shell	*O. edulis* Conway, N. Wales In or on oyster	*C. virginica* Ellerslie, P.E.I. On shell	*C. virginica* Ellerslie, P.E.I. In or on oyster	*O. belcheri* Karachi, Pakistan On shell	*O. belcheri* Karachi, Pakistan In or on oyster
Suctoria									
Acineta tuberosa foetida Maupas (120, 143)	—	R	X	—	—	—	—	—	—
Sphaerophrya solitiformis Lauterborn (74)	—	—	—	—	—	R	—	—	—
Gastrotricha									
gen. et sp. incert.	R	C	X	—	—	C	X	—	—
Rotatoria									
Diplax sp.	—	—	X	—	—	C	X	—	—
Nematoda									
gen. et sp. incert.	C	C	X	—	—	C	—	—	—

TABLE II

Microorganisms recorded from the body surfaces and interior of *Ostrea edulis* (all sources)
(C = common, R = rare)

Microorganism	Healthy oysters		Gapers		Dead oysters	
	Surfaces	Interior	Surfaces	Interior	Surfaces	Interior
Bacteria						
Spirillum sp.	—	R	C	C	C	C
Sphaerotilus dichotomus	—	—	C	R	C	—
Cristispira balbianii	—	C	C	R	—	—
Protozoa						
Mastigophora						
Bodo edax	—	—	R	—	—	—
Bodo rostratus	—	—	R	C	C	C
Dallingeria drysdali	—	—	R	R	C	C
Glenodinium foliaceum	—	C	—	—	—	—
Hexamita inflata	—	—	R	C	C	C
Monas guttula	R	—	—	—	—	—
Monas minima	—	—	C	—	R	R
Prorocentrum micans	—	C	—	—	—	—
Rhynchomonas nasuta	R	—	—	—	—	—
Trepomonas agilis	—	—	C	R	—	—
Sarcodina						
Amoeba radiosa	C	—	—	—	—	—

TABLE II (*Concluded*)

Microorganisms recorded from the body surfaces and interior of *Ostrea edulis* (all sources)
(C = common, R = rare)

Microorganism	Healthy oysters		Gapers		Dead oysters	
	Surfaces	Interior	Surfaces	Interior	Surfaces	Interior
Ciliata						
Ancistrocoma pelseneeri	R	—	—	—	—	—
Aspidisca costata	—	—	C	R	—	—
Chilodonella cucullulus	—	—	C	R	—	—
Cinetochilum margaritaceum	—	—	R	—	—	—
Cohnilembus verminus	—	—	C	C	C	C
Colpidium campylum	—	—	C	C	—	—
Colpidium colpoda	—	—	C	C	—	—
Cyclidium glaucoma	—	—	C	R	R	—
Enchelyodon sp.	R	R	C	C	C	R
Euplotes charon	—	—	C	C	—	—
Holosticha kessleri	—	—	C	R	—	—
Lionotus fasciola	—	—	C	—	R	—
Pleuronema marinum	—	—	C	C	C	C
Uronema marinum	—	—	C	R	C	—
Urostyla gracilis pallida	—	—	R	C	—	C
Unidentified tetrahymenids	—	—	C	C	C	C
Suctoria						
Acineta tuberosa foetida	—	—	C	—	R	R
Gastrotricha						
gen. et sp. incert.	—	—	C	—	C	R
Rotatoria						
Diplax sp.	—	—	—	—	R	—
Nematoda						
gen. et sp. incert.	—	—	R	—	R	R

TABLE III

Microorganisms recorded from the body surfaces and interior of *Crassostrea virginica* (C = common, R = rare)

Microorganism	Healthy oysters		Gapers		Dead oysters	
	Surfaces	Interior	Surfaces	Interior	Surfaces	Interior
Bacteria						
Spirillum sp.	—	R	C	C	C	C
Cristispira balbianii	—	C	C	R	—	—
Protozoa						
Mastigophora						
Bodo edax	—	—	C	—	—	—
Bodo rostratus	—	—	R	C	C	C
Dallingeria drysdali	—	—	—	—	C	C
Hexamita inflata	—	—	R	C	C	C
Monas minima	—	—	C	R	C	—
Ciliata						
Aspidisca costata	—	—	C	—	—	—
Cohnilembus verminus	—	—	C	C	C	C
Colpidium campylum	—	—	C	R	C	R
Cyclidium glaucoma	—	—	C	C	C	C
Enchelyodon sp.	—	—	C	R	C	—
Euplotes charon	—	—	—	—	—	—
Orchitophrya stellarum	—	—	C	C	C	C
Platynematum sociale	—	—	R	C	C	C
Pleuronema marinum	—	—	C	R	C	C
Urocentrum turbo	—	—	C	C	R	—
Uronema marinum	—	—	C	C	C	C
Uronychia transfuga	—	—	C	C	—	—
Urostyla gracilis pallida	—	—	C	C	C	C
Unidentified tetrahymenids	—	—	—	—	—	—
Gastrotricha						
gen. et sp. incert.	—	—	C	—	R	R
Rotatoria						
Diplax sp.	—	—	R	—	R	—
Trematoda						
Bucephalus cuculus	—	—	—	R	—	—

The Ellerslie record (July 7, 1958) for *Bucephalus cuculus* is the most northerly one thus far for this trematode, and the first one for Canada. *O. belcheri* and Karachi constitute new host and locality records for bucephalid cercariae, and the life history of the Pakistan trematode merits local investigation.

Experience elsewhere does not suggest that *Bucephalus* is linked with Prince Edward Island's epizootics. Neither is the presence of *Ancistrocoma pelseneeri* on some European oysters regarded as pertinent to the mortalities at Sam Orr Pond. The usual host, *Mya arenaria*, occurs there, and a tendency to disperse to other molluscs sharing the biotope (70) is in accord with the habits of this ciliate, which has never been regarded as injurious. Of greater significance is the identification of *Orchitophrya stellarum* from two Malpeque Bay oysters. While the observed incidence (7%) was low, both infections of the alimentary tract were heavy and invasion of the intestinal epithelium had taken place. These facts are in accord with Richardson's (124) findings, and it should be noted that incidence of the parasite among male starfish in Connecticut (3) is of similar magnitude (approximately 9%). Up to 22% incidence has been reported from Long Island Sound (52), and spring surges touch 28% in England, where a high level of parasitaemia has been directly related to good feeding (141). The epidemiology of *Orchitophrya stellarum* infections requires further investigation in the light of these data, with attention to the possibilities that the organism is a regular and perhaps harmful parasite of oysters, and that starfish derive their infections from these animals.

Several of the characteristically free-living organisms mentioned in Table I, or closely allied ones, have already been recorded from oysters. Unfortunately, many investigators have disregarded microscopic animals. Sewage bacteria have been mentioned earlier, and Hornell's reference to a fungus-like organism from oysters dying in the 1877–78 epizootic at Arcachon (56) is also suggestive of *Sphaerotilus* or related forms. In addition to *H. inflata*, Certes* (16, 17) found *Prorocentrum micans* and *Enchelyodon* sp. in the water of *O. edulis*, and sketched two ciliates which cannot be identified with certainty. *P. micans* is commonly ingested by oysters and other lamellibranchs (36, 93, 117). However, water blooms resulting from huge concentrations of this dinoflagellate are a possible cause of abortion in oysters and other larviparous bivalves (78). Nelson (102) illustrated (his Plate IV) several organisms which he observed developing rapidly in small containers in hot weather, when decomposing spat furnished them with suitable food. His Figs. 2 and 3 show a uninucleate ciliate (not *Amphileptus* as he thought probable, members of this genus being binucleate) which might well be *Cohnilembus verminus*, a common meso- to poly-saprobic marine ciliate (62). He also illustrated gastrotrichs (Figs. 4, *a–c*) and the subject of his Fig. 6 was probably the predacious ciliate, *Lacrymaria coronata*. Ciliates on Nelson's Plate V, "Microscopic parasites and enemies of oysters", include Spirotricha close to *Euplotes charon* (Fig. 12, *a*, *b*) and *Aspidisca* sp. (Fig. 13). Orton (113) pointed out that ciliates are very common in weakening oysters, their numbers increasing enormously as the death of the host approaches. He found a suctorian (*Acineta*

*His results were contemporaneously reported in the United States by Ryder (129).

tuberosa foetida), common in decaying seaweed (143), in a maceration of oyster heart in sea water (120), and reported that dying European oysters yielded *Colpidium colpoda*, *Glaucoma scintillans*, and (*Loxophyllum*) = *Lionotus lamella*, besides *Aspidisca* sp. and a *Uronychia*-like organism. However, Orton concluded that all these protozoa were merely secondary invaders equally liable to attack any other weakly or dying marine animal.

It is submitted that the obvious logicality of this conclusion blinded Orton to the real significance of his microorganisms as biological indicators of pollution, a significance that cannot be properly appreciated until they are considered as units and groups in a modified version of the "Saprobiensystem" of Kolkwitz and Marsson (67, 74, 135).

The Microbiology of Polluted Waters

The Saprobiensystem, as originally conceived and subsequently expanded by its authors (67), listed numerous species of plants and animals, most of them microorganisms, considered characteristic of the different levels of organic pollution. Three zones of pollution were defined, oligosaprobic, mesosaprobic, and polysaprobic. Under the first, the oxidation of polluting substances is all but complete and vigorous disintegration processes have died away. Mesosaprobic conditions prevail during most of the period of oxidation. Under β-mesosaprobic conditions the ammoniacal compounds of the fatty acids are evident, and the fouler α-mesosaprobic zone is marked by the prevalence of amino acids. Polysaprobic conditions, dominated by the abundance of complex organic matter, are those of heavy pollution and decomposition, with reduction of the polluting substances proceeding vigorously. While this is the classification now adopted, it should be mentioned that certain modern investigators (135) prefer to subdivide the polysaprobic zone too.

Liebmann (74) revised the Saprobiensystem, with greater attention to the life requirements and abundance of the chosen indicator species. Studies of protozoan communities by Picken (118), Fauré-Frémiet (34), and others have also stressed the importance of a broad ecological approach. Recent applications of the system (135) characterize the different levels of pollution by indicator communities of dominant organisms instead of by long lists of species which more often than not tolerate more than one zone. The author (72) has summarized earlier research on the succession of communities of microorganisms as the level of organic pollution changes, and Bick (7) provides quantitative data on such successions.

By its nature less precise than chemical and physical procedures used in water analysis, and primarily intended for application to organically rich stretches of streams, the Saprobiensystem nevertheless lends itself to studies of all kinds of water bodies. Biological balances involve the interplay of so great an array of factors, and tend to be so briefly static, that bioassay methods, used with proper discretion, can form valuable tools for the ecologist. As long as the emphasis is on communities and due attention is given to successions, the Saprobiensystem provides a valid general estimate of organic pollution, and not necessarily of faecal pollution, although it has seen most use in this regard. The microorganisms of biological flocs due to sewage are equally characteristic of films at any mud water interface in natural waters. They

concentrate in the porous upper part of the film (71), which varies in thickness according to the rate of accumulation of dead plants and animals and other organic wastes not necessarily linked with human activities. Their communities, classified arbitrarily in accordance with the Saprobiensystem, provide a measure of the degree of micropollution. While highly relevant to the well-being of lamellibranchs, this aspect of the bottom microenvironment is liable to escape detection altogether where estimates of pollution are based upon chemical analysis of the overlying water mass.

TABLE IV

Status of species in the Saprobiensystem

Microorganism	Zone of occurrence			
	Oligo-saprobic	β-Meso-saprobic	α-Meso-saprobic	Poly-saprobic
Glenodinium foliaceum	X			
Prorocentrum micans	X			
Amoeba radiosa	X	X		
Anisonema acinus	X	X		
Condylostoma patens	X	X		
Keronopsis rubra	X	X		
Lacrymaria coronata	X	X		
Marsupiogaster picta	X	X		
Uronema pluricaudatum	X	X		
Uronychia setigera	X	X		
Uronychia transfuga	X	X		
Holosticha kessleri	X	X	X	
Monas guttula	X	X	X	
Peranema trichophorum	X	X	X	
Tropidoscyphus octocostatus	X	X	X	
Urocentrum turbo	X	X	X	
Aspidisca costata		X	X	
Bodo edax		X	X	
Frontonia marina		X	X	
Lionotus fasciola		X	X	
Platynematum sociale		X	X	
Urostyla gracilis pallida		X	X	
Acineta tuberosa foetida		X	X	X
Chilodonella cucullulus		X	X	X
Cinetochilum margaritaceum		X	X	X
Cohnilembus verminus		X	X	X
Colpidium campylum		X	X	X
Cyclidium glaucoma		X	X	X
Euplotes charon		X	X	X
Oikomonas termo		X	X	X
Pleuronema marinum		X	X	X
Rhynchomonas nasuta		X	X	X
Sphaerotilus dichotomus		X	X	X
Uronema marinum		X	X	X
Bodo rostratus			X	X
Colpidium colpoda			X	X
Dallingeria drysdali			X	X
Hexamita inflata			X	X
Monas minima			X	X
Trepomonas agilis			X	X
Sphaerophrya solitiformis			X	X

The Saprobiensystem in Brackish Environments

Table IV places the species listed in Table I in the Saprobiensystem, which has seen little use in brackish water biology. Data supporting the necessarily arbitrary allocation of individual microorganisms to the four major zones of pollution are to be found in the references included in the latter table. At the higher levels of pollution there is remarkably close correspondence with freshwater conditions, for most of the characteristic polysaprobes are perfectly tolerant of a brackish environment. Among the few that are not is *Colpoda cucullus* O. F. Müller, perhaps the most conspicuous omission from these pages, this species being unable to withstand even the most gradual transition to 10% sea water (35). A familiar β-mesosaprobic ciliate, *Halteria grandinella* (O. F. Müller), is absent too. Although Wailes (143) listed this species from brackish pools on Canada's Pacific coast, Finley (35) declared that *Halteria*, like *Colpoda*, is quite intolerant of even slight salinities. At the other end of the scale, some species, like *Oikomonas termo* (50), can be transferred directly to pure sea water provided that their nutritional requirements are met.

Thus all seven species entered only in the α-mesosaprobic and polysaprobic columns also occur in polluted fresh water or animal macerations. Furthermore, only three (25%) of the saprophiles tolerating polysaprobic through β-mesosaprobic conditions (*Acineta tuberosa foetida*, *Cohnilembus verminus*, and *Pleuronema marinum*) are restricted to brackish waters or the sea; as are three (27%) of the species not found under conditions fouler than α-mesosaprobic (*Frontonia marina*, *Holosticha kessleri*, and *Urostyla gracilis pallida*). The proportion of marine and brackish water species is much higher, though, in the remaining zones. Five (56%) of the oligosaprobic to β-mesosaprobic species (*Condylostoma patens*, *Keronopsis rubra*, *Uronema pluricaudatum*, *Uronychia setigera*, and *U. transfuga*) are limited to the sea or brackish waters, as is one of the two oligosaprobic dinoflagellates, *Prorocentrum micans*.

Significance of Association between Oysters and Normally Free-living Microorganisms

As is evident from Tables I and IV, a single polysaprobic species was recorded from the very thin film of mud on the shells of European oysters at Conway and *Ostrea belcheri* at Karachi. This was *Euplotes charon*, a hypotrichous ciliate found throughout the mesosaprobic zones as well, and known as a facultative parasite of marine invertebrates (sea anemones and echinoderms) (1). A β-mesosaprobic heterotrich, *Condylostoma patens*, present on the valves of oysters at Conway (Table I), has also been reported as a harmless endobiont of sea anemones (1). At the same time, oysters from both localities bore folliculinid ciliates. These plankton feeders are characteristic of oyster shells from clean beds (69), for their tests are quickly blocked by bacterial jelly or accumulating ooze and sediments (2). The nature of their epibionts, and the constant presence of well-formed crystalline styles with abundant *Cristispira*, suggest that both batches of oysters originated in unpolluted waters. They did in fact do so. The Conway specimens had spent a short period in clean tanks associated with *Pecten maximus*, the shells of which likewise harbored few protozoa (*Lacrymaria coronata*, *Urostyla* sp., and unidentified colorless flagellates). They had been placed there following their removal from the Tal-y-foel oysterage

on the Menai Straits, which is at once protected from rough weather by a sandbank and subjected to daily tides (146) which keep pollutants from accumulating. The source of the Pakistan oysters was a harbor-mouth groin at Keamari, Karachi, where tidal scouring is again vigorous and regular.

No sickly oysters were seen at Conway or Karachi, and none of the healthy specimens examined (20 at each locality) bore any epibionts on the surfaces of the body itself. Unlike these individuals, oysters from New Brunswick and Prince Edward Island always had the shell liberally coated with black mud. This excluded *Folliculina*, but provided a substratum for numerous mesosaprobic to polysaprobic organisms (Table I), most of which favor low oxygen levels and tolerate the lowering of pH values accompanying an increase in hydrogen sulphide (60). Studies at Sam Orr Pond proved these organisms representative of the microfauna and microflora of the surface zone of the bottom mud on which, or just above which, rested the oyster trays. Certain inhabitants of the biological film also spread to the gills and other surfaces of the oysters themselves in both Canadian localities (Tables II and III).

Fifty seemingly healthy European oysters were examined at St. Andrews between 1958 and 1960. Thirty originated from Sam Orr Pond, 10 from the 1959 planting at Oak Bay, and 10 from a tray kept in the intertidal zone at the Biological Station wharf. Sickness was never apparent at the second and third sites (28, 95). All examples harbored one or more of four normally free-living protozoons on the gills and mantle. Two of these (*Amoeba radiosa*, *Monas guttula*) are not polysaprobic, one (*Rhynchomonas nasuta*) tolerates all levels of pollution, and the last, *Enchelyodon* sp., was identified from sick and decomposing oysters too. In each case the crystalline style was intact, with abundant *Cristispira balbianii*. The gut flora also included rare examples of *Spirillum* sp., close to if not identical with *S. virginianum* Dimitroff (11, 24), originally isolated from mud on the shell of a marketable American oyster at Baltimore. Polysaprobic preferences are usual in the genus (74), and the same *Spirillum* became abundant in sick European oysters and flourished in dead ones. Other bacteria present appeared to be those of the immediate environment (38).

The only protozoons found alive in the gut of healthy *O. edulis* were *Enchelyodon* sp., already reported from the same host in Europe (16, 17), and two dinoflagellates, *Prorocentrum micans* and the fresh- to brackish water *Glenodinium foliaceum*. The former is widespread in European seas and brackish waters in the spring and summer (19). It occurs abundantly on oyster grounds (131) and in the water of *O. edulis* (16, 17). At times it forms "red water" blooms (119), which can cause complete abortion of embryos and immature larvae by gravid oysters (78, 80). Nevertheless, at optimum concentrations, *P. micans* is undoubtedly an important food organism for oysters (93) and other lamellibranchs (36). Its summer occurrence under brackish conditions in Sam Orr Pond parallels that reported under similar circumstances on the Atlantic coast of the United States (23).

The contents of the digestive gland of healthy European oysters included other identifiable oligosaprobic plankters. Unlike the two dinoflagellates already mentioned, though, these were always dead and sometimes fragmentary. Armored dinoflagellates (*Dinophysis* spp.) less tolerant of brackish con-

ditions than the two species just discussed, and algae of the genus *Chlorella*, were prominent among them. So were β-mesosaprobic diatoms, such as *Nitzschia* spp., which form part of the normal diet of oysters and other filter-feeding marine invertebrates (43, 81), as do various other algae (18, 80) and bacteria of the bottom film (150). Ingested fungal spores and pollen grains, derived from the woods bordering the inlet, were sometimes common too.

As mentioned earlier, seven of these European oysters had *Ancistrocoma pelseneeri* on their gills, although in smaller numbers than did associated soft-shelled clams. The gills and palps of the latter occasionally exhibited scattered trichodonid ciliates as well, and *Urostyla* sp. was recorded once. The dominant molluscs of the Sam Orr Pond basin are quahaugs (*Mercenaria mercenaria*), common periwinkles (*Littorina littorea*), *Macoma balthica*, and the eastern mud snail (*Nassarius obsoletus*) (28). None of those examined bore any microorganisms on the body surfaces. In the gut of quahaugs and periwinkles, diatoms predominated over dinoflagellates (species of *Dinophysis*, *Glenodinium*, *Peridinium*, and *Prorocentrum*). Diatoms were likewise abundant among teeming bacteria in the gut contents of *Nassarius obsoletus*, a predator and scavenger.

European oysters from Sam Orr Pond differed from the indigenous molluscs with which they were associated in exhibiting the mortalities referred to in the Introduction. Twenty-seven species of microorganisms were present upon the body surfaces of the 40 gapers examined, more than five times the total for healthy oysters. All but three of the ciliates and *C. balbianii* were also found in the biological film over the substratum and the mud on the *O. edulis* shells (Table I). They included some two-thirds of the species collected from these sites. Of those not present on or within the oysters themselves, none are polysaprobic, and some (e.g., *Amoeba radiosa* and *Anisonema acinus*) probably prefer the rather higher pH values of a biological film in direct contact with alkaline brackish waters.

Nineteen of the species occurring on the gills and mantle of gapers were also found in the digestive gland, and three of them in the tissues. Gapers lacked a crystalline style, and any rare *Cristispira* were widely dispersed throughout the gut contents and over the external surfaces (113). The spirochaetes were in fact most plentiful on the body surfaces when present at all.

Fifteen normally free-living microorganisms common on the gills and mantle of gapers and identified to the species level (Table II) included one oligosaprobe to α-mesosaprobe (*Holosticha kessleri*) and two β- to α-mesosaprobes (*Aspidisca costata* and *Lionotus fasciola*). The remaining 12 comprised two-thirds of all the mesosaprobic to polysaprobic species encountered. All 11 microorganisms common in the gut or tissues of gaping European oysters (Table II) tolerate or prefer polysaprobic conditions.

Twenty dead European oysters were handled, ranging from examples in which portions of the ciliated epithelium were still feebly flickering to others that were mere blobs of putrefying matter. The number of microorganisms recorded from their surfaces had decreased from the 27 for gapers to 17, the only addition to the fauna being a saprobic rotifer of the genus *Diglax*. Eleven species were identified, all of them α-mesosaprobic to polysaprobic. Finally, 13 of the microorganisms present on the exterior of these decomposing oysters were found in the interior too, eight of them commonly.

Downer (28) reported a cumulative mortality of 75% for a batch of the 1958 stock kept in a tank at the Biological Station. These European oysters exhibited heavy *Hexamita* infection, as did a giant scallop, *Placopecten magellanicus*, associated with them. The flagellate was also found in an American oyster that died in this tank, the bottom mud of which yielded scattered trophozoites of *H. inflata* towards the end of summer.

Turning now to Ellerslie and American oysters, 10 apparently healthy individuals, 10 gapers, and 10 dead ones were examined (Table III). Lack of time precluded paying attention to any associated molluscs or to the mud near the culture trays. The apparently healthy group bore no microorganisms on the gills or mantle, and the only ones found in the gut were rare examples of *Spirillum* sp. and abundant *Cristispira balbianii* in the crystalline style.

The body surfaces of gapers, though, harbored 21 microorganisms, some three-quarters of them recorded from mud on the shell (Table III). As in European oysters the crystalline style disappeared early, its *Cristispira* dispersing through the gut and becoming common on the gills and mantle. Besides the two parasites mentioned earlier (*Orchitophrya stellarum* and *Bucephalus cuculus*) the endobionts included various combinations of 14 of the 20 normally free-living species present on the body surfaces.

Thirteen of the species identified were common on the gills and mantle of gaping American oysters (Table III). They included one oligosaprobe to β-mesosaprobe (*Uronychia transfuga*) and four β- to α-mesosaprobes (*Aspidisca costata*, *Bodo edax*, *Platynematum sociale*, and *Urostyla gracilis pallida*). The remaining seven, and the four microorganisms not specifically identified, all figure in the mesosaprobic to polysaprobic group recorded from gaping European oysters at St. Andrews.

The number of different microorganisms found on the surfaces of dead *C. virginica* had decreased from the 21 for gapers to 16, the only addition to the fauna being the polysaprobic *Dallingeria drysdali*. As was the case for European oysters, 11 species were identified. Apart from the occurrence of scattered examples of *Uronychia transfuga*, and the absence of *Sphaerotilus dichotomus* and *Acineta tuberosa foetida*, the list agrees with the equivalent one for *O. edulis*.

Twelve of the bacteria and protozoa present on the exterior of dead American oysters occurred in the gut or tissues too. Ten of them were classified as common, *Pleuronema marinum* and the nine so listed from the interior of dead European oysters too: *Spirillum* sp.; three flagellates, *Bodo rostratus*, *Dallingeria drysdali*, and *Hexamita inflata*; and five ciliates, *Cohnilembus verminus*, *Colpidium campylum*, *Cyclidium glaucoma*, *Euplotes charon*, and an unidentified tetrahymenid.

Microorganisms Invading Living Tissues

Living tissues were invaded by only three protozoons, *Hexamita inflata*, *Urostyla gracilis pallida*, and *Holosticha kessleri*. The first two were recorded from American oysters, and all three in European ones.

Neither of the ciliates is primarily a bacteria feeder or a carnivore. *H. kessleri* ingests diatoms and detritus as well as bacteria (148), and members of the genus *Urostyla* are all but omnivorous (130). However, on several occasions

both were observed to have followed *Hexamita* in invading the adductor muscle of the host. Their cytoplasm soon became crammed with ingested myolytic spindles, which, as previously described from sickly oysters (89, 113), were plentiful in the muscle tissue of gapers of both species.

Hexamita inflata

The infrequency with which *H. inflata* could be found in the biological film on mud near the oysters' trays and on their shells is in accord with the scarcity of field records for the flagellate. Nevertheless, Certes (16) found it not only in European oysters but in the surrounding brackish water too, and an earlier report from the Atlantic coast of North America (71) identified *Hexamita* sp. from bottom samples, debris, and centrifuged sea water. Although *H. inflata* has wide tolerances, population explosions are obviously restricted to rather a narrow range of conditions. A strongly reducing environment is not the only prerequisite for its appearance in large numbers. Though Liebmann (74) asserted that it occurs regularly and abundantly in almost every putrefying fluid, several of those who have studied sewage effluents and aging infusions have failed to find it. Perhaps, while occurring only in small numbers in alkaline (pH 7.2 to 8.4) brackish waters, it suddenly flourishes under polysaprobic conditions as the pH falls below a critical point in the acid range? During the present studies, its trophozoites certainly teemed, overshadowing associated microorganisms, at pH levels near 5.0.

All members of the genus *Hexamita* Dujardin being extremely small, and their most obvious features being the generic ones—the occurrence of two anterior nuclei, two axostyles, and six anterior and two posterior flagella—size and shape have been the chief criteria employed in differentiating the species from one another. Dujardin (30), who failed to distinguish the full complement of flagella, described *H. nodulosa* as the genotype. Bütschli (13) was probably correct in regarding this as merely a form of the second species established by Dujardin, *H. inflata*. Both exhibit numerous small vacuoles and refractive globules, the former being from 12 to 16 μ long, having small nodules which render its contours irregular, and appearing bifid posteriorly due to the cytoplasm being drawn out into tails along the trailing flagella. *H. inflata* was described as broadly oval, 17 to 20 μ in length, and posteriorly truncate, the hind margin being slightly concave between the points of exit of the widely spaced trailing flagella. The last-mentioned feature is highly characteristic and is clearly illustrated by Mackin *et al.* (89), whose identification was to the generic level only.

One hundred living trophozoites (from European oysters from Sam Orr Pond) were 11.5 to 18.9 μ in length and 6.8 to 11.7 μ in width (av., 14.4 by 8.5 μ). Their trailing flagella measured from 18.9 to 33.4 μ (av. 26.1 μ), being usually about twice as long as the body. By phase contrast many tiny vacuoles and glycogen globules were apparent, the axostyles showed clearly, and in a few especially favorable preparations viewed under oil a fluid appearance of the cytoplasm at the anterior extremity was suggestive of a cytostome. Fixed and stained examples showed shrinkage, measuring from 8.3 to 14.4 μ by from 4.9 to 10.3 μ (av., 11.7 by 6.6 μ). The dimensions of living trophozoites overlapped the ranges of *H. nodulosa* and *H. inflata*, the smaller ones, recent

products of binary fission, often being of less regular outline than the others, the plump and posteriorly truncate appearance of which was in complete agreement with the latter species.

H. inflata was never common on the body surfaces of gapers, but its trophozoites occurred in huge numbers in the digestive gland, where Drinnan (29), too, observed them most often. *Trepomonas agilis*, another facultative anaerobe, sometimes accompanied it, these two flagellates frequently associating according to Kent (65). Infection soon spread to the gonad and blood vessels. As Orton (113) indicated when accounting for the presence of small flagellates (*Bodo* sp.) in the pericardium of gapers, this organ communicates with the exterior by way of the renopericardial canal and urogenital aperture, and relaxation of these passages in sickly oysters would permit microorganisms to pass from the mantle into the pericardium. The common invasion of blood by *Hexamita intestinalis*, a gut parasite of amphibians, is further evidence of the ease with which these flagellates penetrate one organ system from another.

As usual (89), *Hexamita*-containing leucocytes became concentrated in the blood channels, but there was no evidence of multiplicative stages within these cells corresponding to Davis' (21) intracellular schizonts of *H. salmonis*. Like Certes (16, 17), I observed only one method of multiplication, binary fission. There were no grounds for postulating that the presence of more than one rounded-up *Hexamita* in an individual leucocyte was due to anything other than phagocytosis. The ingestion of relatively large, active, and resistant trophozoites might well inhibit the normal migration of phagocytes from efferent blood vessels through surrounding tissues and the epithelial layers to the exterior (89, 138).

As death approaches, there is widespread necrosis of tissues surrounding the blood vessels, digestive gland, and intestine (89). This, the formation of trophozoite-packed abscesses, and the early invasion of the adductor muscle, all suggest the possibility that these flagellates secrete proteolytic enzymes. Having spread through the body, they continue to undergo binary fission and retain their normal activity for some time after the host's death. Indeed, they not only still teemed, but dividing individuals could still be found, in the putrefying remains of oysters dead for 24 hours. Such behavior, while to be expected of *H. inflata*, is hardly in accord with that of a specific parasite; neither are the facts that a giant scallop in a *Hexamita*-infested laboratory tank became as heavily involved as the oysters with which it was associated, and that the flagellate has also been recorded from quahaugs—not only from gapers, but from the liquid remains of a long-dead individual too (p. 474).

Numerous species of *Hexamita* have been described as internal parasites of insects, myriapods, fish, amphibians, reptiles, birds, and mammals. One has even been recorded from a trematode itself endoparasitic in eels (57). Nevertheless, specific parasitism must not be too hastily assumed where the host is aquatic and living under congested and otherwise abnormal conditions. Even if *Hexamita salmonis* (21) is an obligate parasite of trout transmitted, as claimed by Moore (97), via the egg, it does not follow that all *Hexamita* associated with hatchery epizootics or thriving in dead trout (144) are necessarily referable to the same species. Significantly, Davis (21) concluded that the factors most conducive to the development of *H. salmonis* are overcrowding

and underfeeding of the host, an unsuitable water supply, and oxygen deficiency. Pending morphological evidence to the contrary, the possibility of facultative parasitism by *H. inflata* should always be entertained, whether the aquatic host in question harbors its flagellates internally (144) or externally (109).

The Protozoan Community of Putrefying Oysters

The time of death of oysters defies normal definition, for microscopic examination may disclose active ciliated cells in individuals in advanced states of decomposition. For present purposes, though, a persisting gaper no longer manifesting co-ordinated life processes and clearly beyond any hope of resuming them is considered dead. At this stage several acidophilic protozoons, previously restricted to the body surface and gut contents, spread to the tissues. Notable among them are tetrahymenid ciliates, packed with refringent food spherules and conforming to the description of *Glaucoma scintillans* Ehrenberg. Recent work has shown the undesirability of positively identifying tetrahymenids without silver impregnation, which was not employed in this investigation. The determination is accordingly left open, although these ciliates cannot be differentiated from those found in polysaprobic liquids by several workers and assigned by them to *G. scintillans*. The species, which Orton (113) reported from the gut of dying oysters, has been identified from waters of a hydrogen ion content as high as pH 7.6 (7), but is best known from acid media. Kidder (66) gave its pH range as 5.6 to 6.8 and Šrámek-Hušek (135) recorded it at pH 6.1.

The other ciliates still prominent in both hosts as putrefaction proceeded were *Cohnilembus verminus*, *Colpidium campylum* (which, according to Kidder, grows best at pH 5.4), *Cyclidium glaucoma*, and *Euplotes charon*. This is evidently a highly characteristic community in decomposing organic matter in brackish water, for all its members were also common in secondarily infected external lesions on fish from such water at Singapore (original data, unpublished).

The bacteriophagous *Colpidium campylum*, *Euplotes charon*, and tetrahymenids remained present for 48 hours. By then *H. inflata* could no longer be found, the only flagellate left being a species characteristic of animal macerations, *Dallingeria drysdali* (65). Using nitrazine paper a pH value of approximately 5.0 was obtained from the putrefying remains. Hunter and Linden (59) gave figures of pH 4.6 to 5.0 for similar material, the bacterial flora of which they described in detail.

Synopsis of Microorganisms Associated with Oysters

To sum up, healthy oysters were found in the presence of oligosaprobic plankters and a variety of microorganisms of wide pollution tolerances. While their metabolism remained normal, they were unaffected by the proximity of saprobic bacteria and protozoa inhabiting the biological film covering their shells and the substratum. Relaxation of the adductor muscle, resulting from environmental deterioration, led to the appearance upon and within their bodies of many of the more acidophilic mesosaprobes and polysaprobes from the surrounding film. The community of microorganisms that now developed was characterized by well-known indicators of organic pollution. These

included "sewage fungus" (*Sphaerotilus dichotomus*), bacteriophagous and saprozoic colorless flagellates, and bacteriophagous (*Aspidisca costata*, *Chilodonella cucullulus*, *Cohnilembus verminus*, *Colpidium* spp., *Cyclidium glaucoma*, *Euplotes charon*, *Uronema marinum*, and tetrahymenids) and carnivorous (*Enchelyodon* sp., *Lionotus fasciola*, and *Uronychia transfuga*) ciliates—a community much resembling Picken's (118) "fungus association".

The rapid evolution of this complex community paralleled that of the bacterial flora forming the basis of the food chain. Those of its members with the least oxygen demand and highest acid tolerances were able to spread into the alimentary tract, with the continued fall in the resistance of oysters becoming progressively weaker under continuing anaerobic conditions. At some as yet undefined critical point, lowered host resistance led to the invasion of the blood and tissues by *Hexamita inflata*. With the exception of two ciliates (*Holosticha kessleri* and *Urostyla gracilis pallida*) that were sometimes associated with the flagellate in the adductor muscle, *H. inflata* was the only protozoon to enter the tissues before death took place. Afterwards, the liquefying remains of the host nourished a highly polysaprobic community comprising myriads of bacteria and large numbers (but relatively few species) of protozoa.

Unexplained mechanisms in the succession include the means whereby *H. inflata* overcomes host resistance and penetrates living tissues, and the relationships between it and ciliates joining in the invasion of living and dead tissues. The latter protozoa soon give place to bacteria after the disappearance of *H. inflata*, suggesting the possibility that this flagellate, like *Oikomonas termo* (51), renders conditions tolerable for certain ciliates by detoxifying bacterial metabolic products.

An Ecological Background to Oyster Mortalities

Such a cycle need not necessarily run its full course. It may be arrested or reversed if environmental conditions improve before the molluscs have lost the ability to resume normal metabolic activity, although whether they can, in fact, recover once the blood has been invaded by *H. inflata* is not known. Nor must its effects be uniform over wide areas or even over individual oyster bottoms. While the accumulation of excretory products and the excessive growth of microorganisms during periods of stagnation inevitably affect oysters adversely (10), mortalities are likely to be most pronounced where accumulations of organic pollutants are heaviest. Environmental pressure of this kind is part of the usual hazard of existence, occasioning continual scattered deaths just as do parasites and predators in balance with host populations. After all, cumulative mortalities of 10 to 20% are regarded as normal by oystermen (76, 113). Alarm is only occasioned by sudden rises above such levels, as at Sam Orr Pond between July 16 and 24, 1959. The cumulative mortality for 2-year-old and 3-year-old European oysters then jumped from 9% and 2.7% to 44.6% and 11% respectively, and all gapers teemed with *Hexamita*, which was much less evident both before and afterwards (28).

It is submitted that events like this are initiated by interruptions of the normal feeding activities of oysters, brought about by the high oxygen demand of pollutants, associated with a sudden rise in the level of bacterial activity in

the immediate vicinity. The numbers of saprobic organisms of all kinds rapidly increase, the rate of pumping decreases correspondingly (80), and shell closure is forced as a strongly reducing anaerobic environment develops (83).

At Sam Orr Pond, the initiating stimuli are linked with the facts that an entrance bar prevents tidal flushing during the neap phase, and that winter freezing takes place. During the neap phase, the oxygen level quickly falls to zero in the bottom zone of the basin. Thus on August 4, 1959, after an estimated 10 days without any flushing, no oxygen could be detected there, and on the 31st of that month, after only 6 days without flushing, the level had already fallen to 2.5 cc per liter (28). At these times, disturbance of the mud produced a strong smell of hydrogen sulphide. An ecological survey revealed patchy deposits rich in organic matter (28), the decomposition of which was accelerated by summer temperatures. A frequently overlooked factor contributing towards reducing activity in such deposits is that of self-silting by bottom-dwelling animals. Faeces and pseudofaeces, if not removed by external forces, accumulate rapidly enough to bury a single layer of oysters within 36 days (82, 83), and the annual wet weight of self-silt discharged from a single Japanese oyster raft has been estimated at 6 to 10 tons (60). A high percentage of this material being organic in origin, large amounts of free energy become available to microorganisms, and local bacterial decomposition is greatly accelerated.

There is a rapid decrease in the salinity of the surface layer of Sam Orr Pond during periods of stagnation. On August 24, 1959, immediately after flushing, the surface salinity was 29.3‰ (84% sea water), by comparison with a bottom reading of 31.3‰ (89% sea water). Two days later it was 28.3‰, on the 28th 17.5‰, and on the 31st only 15.1‰ (43% sea water). On the last-mentioned date, however, the bottom salinity stood close to the earlier figure, at 31.2‰ (28). Flushing following such a sequence has rapidly fatal consequences for purely marine plankters. From time to time macroplankters such as hydrozoan medusae and other coelenterates become very numerous in coastal waters, and jellyfish, among the more obvious marine invertebrates entering Sam Orr Pond on spring tides, quickly perish in the surface layer of too brackish water. Mortalities of this nature are less spectacular than the rotting of piles of stranded seaweed, which have been said to release gases directly toxic to oysters (126), but their significance is just as great. They cancel the temporary advantages — raising of the oxygen level and replenishment of the supply of oligosaprobic food organisms — derived by the oysters from tidal flushing of the inlet. With the onset of intense bacterial activity in the decomposing remains of macroplankters, the local environment again becomes a strongly reducing one. Once more the oysters must close their shells frequently, feed only sporadically, and cleanse themselves irregularly.

Any food intake in anaerobic periods must be rich in bacteria, and each interruption of feeding leaves great numbers of these not only throughout the gut but also over the body surfaces, particularly about the strings of mucus which normally transport food over the gills to the mouth (87). Oysters debilitated by pollution have the gills clogged by this mucus (107). As weakening of the adductor muscle from undernourishment and allied causes prevents proper closure of the shell valves, and gaping heightens exposure to predators

and infection (134), the bacteria-rich mucus mat over the gills invites invasion by bacteriophagous protozoa. Many of the microorganisms of the surrounding biological film thus move into this niche, some of them spreading to the digestive tract and tissues as already outlined. The ability of oysters to survive direct effects of an anaerobic environment (113) must inevitably break down should such conditions be too long maintained.

There is circumstantial evidence that they had been too long maintained at Sam Orr Pond just prior to the heavy mortalities of July, 1959. Downer (28) estimated that after June 27, 18 days went by without any flushing, the stagnant period culminating in prolonged anaerobism and overly high water temperatures. On July 16, Downer found that many oysters, although not yet gaping, felt "springy" when squeezed, indicating that the adductor muscle could no longer close the shell valves completely. That the subsequent death rate of 2-year-olds was four times that of 3-year-olds probably reflects earlier relaxation of the less developed adductor muscle in the younger oysters, leading to their longer exposure to the hazard of hexamitiasis—for the more efficient the valve closure of lamellibranchs during a period of acute danger of invasion by saprobic organisms, the better their prospects of surviving an anaerobic spell. Significantly, quahaugs were not involved in the outbreak. *Mercenaria mercenaria*, the most plentiful lamellibranch associated with the infected oysters, avoids undue bacterial invasion through being able to close its shell extremely tightly (27), and it will be remembered that even under the congested conditions of a laboratory tank where *H. inflata* occurred alike in European and American oysters, a giant scallop, and the bottom mud, the single quahaug present remained uninfected (28). Nevertheless, although able to resist infestation with microorganisms longer than oysters can, quahaugs are no less vulnerable to *Hexamita* once they are forced to gape. Mr. R. E. Drinnan (personal communication) has recorded the flagellate from three of them from Neguac, New Brunswick, two being lightly infected gapers while the third was reduced to a putrid liquid teeming with *Hexamita*.

With the onset of winter, bacterial decomposition proceeds in the self-silt that accumulates until water temperatures fall to a level (about 4° C) below which European oysters cease to cleanse themselves (25). The molluscs again become subject to the hazard of invasion by microorganisms, which takes place just as in summer if the adductor muscle begins to relax, as happens when sustained reductions of temperature to too low a level lead to loss of vitality (127). Low environmental temperature and not oxygen deficiency was held primarily responsible for the gaping of European oysters under the ice covering Sam Orr Pond in the winter of 1958–59 (28). Specimens then examined showed as heavy hexamitiasis as any seen in the summer, a very similar situation having been reported from Dutch holding basins, or pits (89). Once again, associated lamellibranchs remained free from facultative parasites. Quahaugs have already been discussed in this regard, and the other species examined, the soft-shell clam, is known to be able to cleanse itself of microorganisms at temperatures near freezing point (92).

The 100% mortality of European oysters at Ellerslie during the winter of 1958–59 was, as at Sam Orr Pond, associated with high incidence of *Hexamita* and temperatures of, and a little below, 0° C (29). Despite the severity of the

winter, equivalent mortalities did not take place among Prince Edward Island's native American oysters. Perhaps they escaped involvement through their capacity to continue normal metabolic activities at somewhat lower environmental temperatures than can *O. edulis* (39), and because of the superior efficiency of their cleaning mechanism (108).

Mackin *et al.* (89) reported that oxygen was 90 to 98% saturation and organic matter was normal during winter outbreaks of hexamitiasis among European oysters in Dutch holding basins. These facts are not at variance with the concept now presented. Even well-aerated waters support saprobic microorganisms, which are concentrated in a biological film so thin as not to affect the results of chemical assay of organic pollution. If oysters in stasis lose their resistance to invasion through failure to withstand prolonged low temperatures, mucus on their body surfaces is just as likely to attract bacteriophagous and other saprobes as it is in summer. Slower development of climactic polysaprobic communities will, of course, be evident than when the heavier organic pollution of warmer months enriches biological films as at Sam Orr Pond.

Mud thickly coated the American oysters examined at Ellerslie. The rich biological film on its surface proved to carry abundant microorganisms close to those found at Sam Orr Pond, and sickly and dead oysters harbored the same saprobes in both localities. Such evidence of environmental pollution was conspicuously absent when oysters from clean bottoms were inspected during similarly brief visits to Conway and Karachi. It is therefore suggested that future studies of "Malpeque disease" should be so conducted as to exclude any possibility of confusion between deaths due to an unknown pathogen and those simply hastened by facultative parasitism under environmental conditions unsuited to the maintenance of stable oyster populations.

Environmental Pollution and Past Oyster Mortalities

Declines in oyster populations have sometimes been attributed to sewage or industrial wastes, notably pulp-mill effluents. A great deal more notice has, however, been taken of the direct effects of these pollutants upon oysters than of secondary effects upon the microenvironment. Published observations nevertheless permit some speculation about the contribution of the latter to abnormally high death rates.

One of the clearest indications of the association of alarming oyster mortalities with deterioration of the immediate environment through gross pollution concerns the Taranto disaster of 1919–20. Orton (113) quotes Professor Cerruti for the information that these beds became contaminated by sewage effluents from World War I military camps and heavy shipping. The anaerobic conditions that developed were worsened by the release of free H_2S and a heavy silt suspension during a naval salvage operation. This combination of circumstances, itself sufficiently detrimental to oysters (60), terminated in heavy infestation of the latter with filiform bacteria. It is a legitimate assumption that polysaprobic protozoa characteristic of the *Sphaerotilus* association were abundant too.

Industrial wastes have been blamed for weakening the adductor muscle of oysters and causing gaping (107). Galtsoff *et al.* (42) and McMillan (88) both reported that the highest oyster mortalities due to pulp-mill effluents coincided

with the highest sulphate levels, and it is suggested that the biological film at the bottom of these zones of vigorous chemical oxygen demand was characterized by an abundance of *Beggiatoa alba*. This species, another of the sewage bacteria, is very prevalent in sulphate-rich brackish water. It coats mud surfaces with a whitish film the ciliates of which have been studied by Fauré-Fremiet (34). The growth is first exploited by the bacteriophagous *Colpidium campylum* and *C. colpoda*, which attract predaceous ciliates (*Enchelys*, *Lionotus*, *Leucophrys*), an association succeeded by *Chilodonella cucullulus* and tetrahymenids. While low concentrations of pulp-mill effluents stimulate the growth of *Nitzschia* (42) and *Melosira* (88), β-mesosaprobic diatoms in the presence of which oysters develop well, hexamitiasis is thus a likely event when high concentrations favor the development of polysaprobic communities. Schodduyn (131) associated summer losses in a European oyster park with the appearance of a *Beggiatoa* mat.

Fresh-water dilution, too, has had most attention from the standpoint of its direct effects upon oysters. Thus Galtsoff (40) attributed an epizootic in Mobile Bay, Alabama, to a highly unusual coincidence of heavy local rainfall with the flooding of rivers discharging into the Bay. Again, Orton (115) considered high mortalities of *O. edulis* in the British Isles due to the "continuous freshet that might have followed the heavy autumnal rains of 1934", and the death of up to 75% of American oysters in parts of Chesapeake Bay in 1945–46 was ascribed to the fact that salinities had fallen to only one-half to one-third of normal (61). U.S. Fish and Wildlife Service data show five peaks of oyster mortality in upper Chesapeake Bay during the previous 38 years. All were reached at times when heavy rains had caused the Susquehanna River to reach an abnormally high level, salinity in the Bay being much reduced in consequence (4).

As to indirect effects of unduly fresh conditions upon oysters, heavy Australian casualties have been ascribed to bacteria thriving in *Polydora* blisters following the death of the worms through fresh-water exposure (149), and Roughley (127) reported lesions resembling those due to hexamitiasis in oysters subjected to freshets in New South Wales.

Inshore salinity reductions also marked the severe British mortalities of 1920–21. None of the accounts of events of this sort consider the possible contribution to organic pollutants through the wholesale death of fully marine organisms brought in by tides. Yet such plankters are attuned to remarkably stable salinity conditions, and all typically open sea species perish when subjected to drastic reductions in the chloride level (20). So, for that matter, do European oysters, which, although their deaths may be hastened by invading saprobic microorganisms, are highly unlikely to survive extended exposure to an unduly freshened environment. The latter alone largely explains certain notable failures of oyster fisheries. For example, the rapid decline of the Firth of Forth beds in the late 17th century, when European coastal salinities fell due to the rains that coincided with the retreat of the Chamonix glaciers; and the dwindling production from English and Norwegian oyster bottoms during the abnormally wet 1870's (137). *Hexamita inflata* must have flourished at these times and during the British mortalities of 1920–21, but only in the rôle of a scavenger except towards the periphery of its range, where, although

organic decomposition was more than usually pronounced, depth or distance from the shore minimized salinity reductions. There, the flagellate might well have ensured the death of many oysters that could otherwise have survived.

The latter rôle of hexamitiasis is regarded as of most importance to European oysters grown or overwintered under marginal conditions. It is perhaps of even greater significance to American oysters, which are normally cultured in bays and estuaries. Following prolonged rains and flooding, organic material of both marine and fresh-water origin accumulates on estuarine bottoms. *Crassostrea virginica* tolerating the physical effects of a considerable measure of dilution (examples have survived exposure to fresh water for several weeks) is it not conceivable that failure to survive freshets is often due to the activities of pollution microorganisms including *H. inflata*? This suggestion is supported by information supplied to Orton by the then U.S. Commissioner of Fisheries, who wrote (113) that, during the Chesapeake Bay mortalities of 1916, the zone about the dying oysters was anaerobic and unusually rich in decomposing organic debris.

Conclusions

Oysters, like other animals, play host to a variety of true parasites. When they exhibit abnormally heavy mortalities from unknown causes, it is therefore tempting to postulate parasitic scapegoats, the choice of which is influenced by prevailing fashions in research. In the latter nineteenth century a bacterium had to be responsible for "Maladie du pied"; when protozoology held the stage early in our own century it was inevitable that an undiscovered protozoon would be proposed as the possible cause of epizootics in Norwegian oyster ponds (49); and more recently, there have been suggestions that a virus may be to blame. The fact remains that no studies of major oyster mortalities have yet proved any specific parasite of first importance per se (134), and it has been contended that such parasites only assume grave significance when general environmental deterioration so lowers host resistance as to transform regular, low-level loss into disaster (145).

Reference to the extensive literature on the vicissitudes attending the culture of oysters suggests that too often, as in the extreme example of Sam Orr Pond, they are grown under conditions inviting intermittent disasters. After all, the oysters with which this paper is concerned are reef, not bottom, animals (103). Granted favorable temperatures and food availability, they grow best on a hard substratum, not a muddy one (28), in places where little silt can accumulate (22). Optimal sites for both species are just below low-water mark and subject to daily tidal influences, for constant submergence offers the steadiest growth of shore lamellibranchs (22, 146, 147) while regular scouring minimizes pollution hazards.

Such sites are admittedly apt to be exposed to higher levels of predation (147) than brackish waters. If the latter are relatively shallow their summer warmth will combine with an abundance of food material to ensure good growth of oysters physiologically suited to them, and at the same time they provide especially favorable harvesting conditions. American oysters in particular are thus commonly cultured in sheltered estuaries under conditions obviously advantageous to the growers, who, knowing that their unnaturally congested

stock can recover from the direct effects of temporary exposure to physiological adversities encountered there, have become aware of only part of the hazard of pollution in waters of this kind.

There are several roads to the polysaprobic zone, and neither high levels of *Escherischia coli* nor toxic industrial effluents, the two factors usually equated with the term "pollution", are necessarily prerequisite to the development of saprobic communities. Less obvious natural pollution through the accumulation of self-silt or of the remains of organisms killed by sudden salinity changes, is easily overlooked. Analysis of samples from the water mass overlying oyster bottoms can altogether fail to detect pollution of this order, leading to claims that the level of organic matter is tolerable when, in fact, biological assay of the film in the immediate vicinity of the molluscs themselves would show that it is not. Saprobic microorganisms are always present in aquatic habitats. Rotting oysters, like any other organisms dying there, will yield them in abundance even when a high level of cleanliness ensures their relative rarity in the biological film over the substratum (131). It is only when they begin to flourish in the immediate environment of living oysters, that communities of the Saprobiensystem assume significance as biological evidence of pollution, a fact that justifies repetition of a recent warning that those concerned should "make special efforts to examine in all possible detail the factors which interact to make a particular environment more or less suitable for oysters" (12).

It is fully appreciated that the original data presented herein are not only scanty but mainly qualitative. Nevertheless, considering them against the background of a considerable literature, it is believed that they support certain general conclusions:

(*a*) that when temperature, rainfall, or tidal factors render a local environment physiologically unfavorable for oysters, these become progressively less resistant to invasion by saprobic microorganisms;

(*b*) that hexamitiasis results from facultative parasitism by one of these microorganisms, *Hexamita inflata*;

(*c*) that locally anaerobic conditions predisposing to hexamitiasis are indicated by the flourishing of communities of the Saprobiensystem in the immediate vicinity of living oysters and within the shells of sickly individuals;

(*d*) that unless environmental conditions improve before such oysters lose the ability to recover, there will be abnormal mortalities, hastened, though not primarily caused by, the invading microorganisms;

(*e*) that micropollution and hexamitiasis are contraindicated by the abundance of *Cristispira balbianii* in a well-developed crystalline style, and of folliculinid epibionts.

As to the practical significance of these conclusions, it is obvious that nothing can be suggested for the protection of oysterages normally kept clean by tidal flushing and located in narrows or on exposed coasts where dilution can only become a problem through freshets of a magnitude reached perhaps two or three times in a century. Such oysterages, like those near Conway and Karachi, have long trouble-free histories. These are sites where the oyster species concerned thrive best in nature, and only the impact of rare and profound environmental crises is likely to cause major population fluctuations.

If oyster hexamitiasis is indeed simply an end point in a chain of events initiated by deterioration of the environment, there is clearly no general remedy for the condition. Each outbreak poses its own special problems, cycles in the community of the bottom biological film being influenced by the various stimuli described in the preceding pages and undoubtedly by others too, for example periodic tide-induced fluctuations in the concentrations of potassium and other elements, affecting the activity of marine organisms (41). Just as Davis (21) remarked regarding *H. salmonis*, the havoc caused by hexamitiasis is in inverse ratio to the suitability of the environment. Much could be done to render it less hazardous by improving culture techniques. It is timely to recall a remark made long ago by Dr. P. C. C. Hoek and quoted by Korringa (68), namely that oyster culture *is* a culture and not a manufacture.

The results of studies of the tolerances of oysters have tended to lead to the intensive exploitation of areas chosen by growers, rather than towards an understanding of the conditions optimal for these animals in nature. The concentrated research into these conditions urged by a few investigators in recent years (12, 145) has yet to be undertaken, and is again advocated.

Present data suggest that solutions for local problems would include the elimination of overcrowding on oyster beds, the free use of shell to harden the muddy bottom of tidewater ponds (86), and regular removal of self-silt and other deposits of high organic content where conditions favor their rapid accumulation. This could be effected by dredging, already employed with encouraging results in Holland (69), and resorted to in combination with scraping for the removal of mud crabs (the primary hosts of the gregarine, *Nematopsis ostrearum*) from some American beds (53). Rotation of oyster crops and bottom ploughing—of more limited application than suction dredging, being dependent on tidal exposure (60)—also offer possibilities in suitable situations. Improved techniques, particularly for the control of predators, might justify the development of new oysterages on the open coast where micropollution problems are minimal.

Intensive biological research, besides revealing how best to exploit oysters native to particular areas, is the only means of providing a sound basis for the establishment of chosen stocks in new localities. Perhaps other strains of European oysters than the Conway one might prove more adaptable to a polluted and muddy environment. After all, the species has adapted itself to widely differing conditions from Norway to the Aegean, and hybridization and selection could be exploited in this connection (48). Again, oysters differ among themselves as regards the efficiency of valve closure. By analogy with the relative freedom from microbial invasion exhibited by other lamellibranchs associated with European oysters at Sam Orr Pond, as yet unconsidered species of oysters might prove more adaptable to Canadian Atlantic coast conditions. The practicability of using air transportation in effecting new introductions having now been clearly demonstrated, no bar remains to experimenting with candidate species (Australasian mud oysters for example) from the most distant sources.

Acknowledgments

It is a pleasure to record my thanks to Dr. J. L. Hart, Director of the

Fisheries Research Board of Canada Biological Station, St. Andrews, N.B., and to many of his staff who helped in various ways throughout these studies; in particular to Dr. J. C. Medcof, Mr. P. J. Downer, and Mrs. Esther I. Lord, and to Dr. R. R. Logie and Mr. R. E. Drinnan, former and present officers in charge of the Biological Substation at Ellerslie, P.E.I. I am indebted, too, to Mr. P. R. Walne of the Fisheries Experiment Station of the United Kingdom Ministry of Agriculture, Fisheries and Food at Conway, North Wales, and to Dr. A. R. Ranjha, Officer-in-Charge of the Pakistan Ministry of Food and Agriculture Zoological Survey Department, Karachi, Pakistan, for their kindness and ready assistance during my visits to their laboratories.

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(*Reprinted from Nature, Vol.* 189, *No.* 4766, *p.* 774 *only*, *March* 4, 1961)

A Biennial Copepod from Lake Hazen, Ellesmere Island

SEVERAL species of copepods in the Arctic are known to have annual life-cycles in the sea[1] and fresh-water[2], and macroplanktonic Crustacea of northern seas may not breed until two years old[3]. So far as I am aware, no copepod has been described with a life-cycle greater than one year, and the extreme response of *Cyclops scutifer* in Lake Hazen to conditions of low temperature and limited food seems worth recording.

Lake Hazen (lat. 81° 45′ N., long. 71° 00′ W.) is the largest lake of the Queen Elizabeth Islands of high-arctic Canada. It is glacier-fed, cold and deep, and is not always entirely free from ice in summer. Primary productivity is very low, and the fauna is small in species and individuals.

The zooplankton was sampled regularly between mid-June and mid-August. The proportions of naupliar and copepodite stages at one offshore station on four successive dates are given in Fig. 1. The proportions of the stages were very similar in plankton

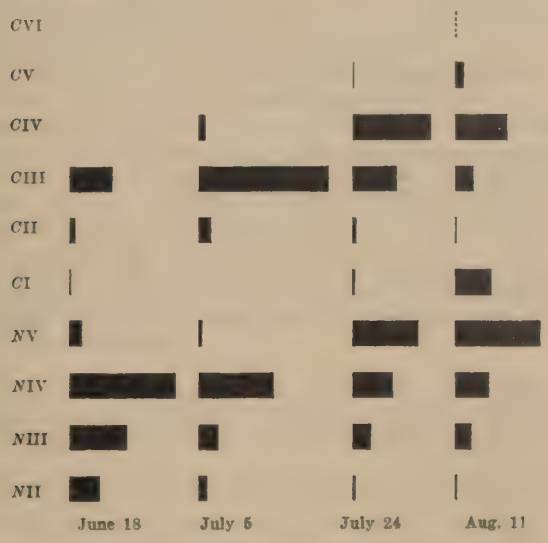

Fig. 1. The proportions of the stages of nauplii and copepodites of *Cyclops scutifer* in vertical plankton hauls from 32 m. at the same station on four successive dates. No adults (CVI) were taken in the vertical haul of August 11, but a few were taken in a nearby surface collection on that date

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collections from other parts of the Lake. The conspicuous and persistent bimodality of the population throughout the short sampling period can only be the result of a basically biennial life-history. A very few adults were taken in a surface haul on August 11, the last date of sampling, but presumably most copepodites do not reach adult-hood and breed until the autumn months. The products of this breeding pass the winter as nauplii and just begin to enter the copepodite stages by the following summer. The possibility of slight exchange between the generations is suggested by the stage I copepodites of June 18 and the stage II copepodites of August 11.

I am indebted to Dr. Edward B. Reed, Colorado State University, for finding the half-dozen adult copepods in the entire collection and identifying the species as *Cyclops scutifer* Sars. Field work was carried out in summer 1958 on the Canadian International Geophysical Year expedition to Ellesmere Island, organized by the Defence Research Board of Canada. Collections were analysed at the Bingham Oceanographic Laboratory, Yale University. A more complete account of the aquatic biology of Lake Hazen is in preparation.

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Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.

DATA CONCERNING PRODUCTION, VALUES, IMPORTS AND EXPORTS, COMPOSITION, NUTRITIVE VALUES AND USES OF PACIFIC OCEAN MARINE PRODUCTS

By H. L. A. Tarr

The tabular information which is presented in the following tables* is extremely general and fragmentary and has been collected merely to assist participants of the Conference in their knowledge of some fishery problem important to the present area of discussion. Most of the data pertains to the United States and Canada, but some information on catch statistics has been included for the U.S.S.R. and Mainland China. These countries even now, and I am certain in the years to come, will reap an increasingly large harvest of Pacific edible fishes. A list of references which were used in completing this report is included.

For simplicity, the various fish classes have been divided into groups in accordance with F.A.O. publications. It is of interest to note that about 90% of the world's fish are obtained in the Northern Hemisphere, but it is likely that future developments may alter this. Neither of the world's two major fish-producing areas, namely the northeastern Atlantic and northwestern Pacific is heavily exploited by United States or Canadian fishermen. Most of the fish taken by north American fishermen are obtained at present from the northeastern Pacific (coasts of British Columbia and Alaska), eastern central Pacific, northwest Atlantic (off Newfoundland) and western central Atlantic. The Great Lakes area and some of the Canadian northern lake areas yield freshwater fish. It is important to note that during the past decade there has been only a minimal increase in the fish landed in North America (from 3.36×10^6 metric tons in 1947 to 3.90 in 1958). In the same time the landings in Asia, including mainland China, have increased from 6.27×10^6 to 16×10^6 metric tons, and in Russia from 1.47 to 2.54×10^6 metric tons. It is clear that in North America we lag far behind in respect to increases in fresh fish landings. The following tables list some of the principal economic data which are available concerning fish for the North American continent, Japan, the Philippines and, where possible, for Russia and mainland China.

Table I shows that production decreases in the following order: Mainland China, Japan, the United States, Russia, Canada and the Philippines. The figure of 6,000 for mainland China is open to serious question

* For additional statistical data see Tables 101-104 in the supplement.

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and has been arrived at to some extent by including an increased domestic fresh-water fishery (pond culture). In any case, over the five years from 1953 to 1958, production in Japan, Russia and mainland China has obviously increased enormously. This means that there will be an increasing competition for fish in the Pacific Ocean and Bering Sea which is already making itself felt in the salmon fisheries.

Table II shows some of the available data for landings of fish which probably all originate in the Pacific Ocean. Some increase between 1953 and 1957 is obvious for the United States and Canada. For Japan, the big increase was in the North Pacific fishery, an increase which was proportionately much greater than that of the "domestic" fishery.

Table III lists the landed value of different groups of fish for the United States and Canada between 1951 and 1957. As observed previously, the United States position has remained almost static; Canadian landings have increased significantly. The only marked single change in the United States landings was the decrease in value of tuna species, and this was largely balanced by increases in the herring, sardine, cod and flounder fisheries. The increase in Canadian landings of salmon species accounted almost entirely for the increase between 1953 and 1958. By way of contrast, landed values for several other prominent fishing countries are appended, and in several instances marked increases between 1953 and 1958 are evident.

Table IV gives an approximate breakdown of the amounts and values of domestic fishery products in the United States for 1957. About 78% of the products are fresh and frozen and canned items, the largest amount being canned.

Domestic Production and Exports and Imports of Fish Products by the United States and Canada

At the time of preparation of this article very recent information concerning economic values has proved impossible to obtain at the comparatively short notice which was given to the reviewer. For this reason the data which will be presented refer largely to 1957 or 1958. Thus, in our library "Fishery Statistics of the United States, 1957" arrived last July and the volume dealing with 1958 data has not as yet been received. This will not surprise those present who are familiar with this type of publication which consists of over 400 pages of tabular and other data and thus represents a rather terrific effort. The Canadian statistics for the year 1957 were published in 1960, but fortunately a recent brief 1959 review was received in April of this year.

Of the reported 1957 United States commercial fish landings of approximately $4{,}800 \times 10^6$ pounds of fish, valued at $\$351 \times 10^6$ to the fishermen, only $\$36 \times 10^6$ worth was exported (about 10%). On the other hand imports, which have increased fairly steadily over the past decade

were valued at 297×10^6. Though obviously a comparison is unfair, it is important to note that the value of fish landed and that of fish imported into the United States is closely similar. Superficially these figures may appear large, but they represent a comparatively small per capita annual consumption of edible fish in the United States of only 10.2 lb. The probable shortage of red meats which may occur within the next two decades could bring about a revolutionary change in this figure and result in a more prosperous domestic fishery. It is interesting to observe that the average price per pound paid to United States fishermen in 1940 was 2.44¢. By 1946 this had risen very sharply to about 7¢, and for over 12 years has remained comparatively static, never rising above 8.17¢.

As indicated in Table V exports of fish from the United States is made up largely of canned fish and shellfish and of marine oils. Comparatively small amounts of fresh, frozen and cured fish are exported.

As previously mentioned, the United States imports large quantities of fish and fish products, both raw and processed (Table VI). Large amounts of fresh and frozen fish of both marine and fresh water origin and of canned fish and shellfish are included in the total quarter of a billion dollars worth of edible fish imports. Miscellaneous by-products of diverse nature (shells, sponges, marine colloidal gelling agents, fish meals and solubles) make up only a little more than one-seventh of the total imports.

Table VII lists the quantities and values of some of the more important fish items imported into the United States in 1957. Nearly all of the frozen tuna came from Japan and, to a less extent, from Peru. Shrimp were imported from Mexico, Panama, Ecuador, Japan, Hong Kong and India. By far the largest supplier of frozen ground-fish and other fish fillets, steaks and blocks was Canada, but Iceland and Japan contributed quite significant quantities. Most of the imported canned tuna (largely canned in brine) was from Japan, and the bonito and yellowtail from Peru.

Canadian Exports and Imports of Fish

From 1947 to 1951 there was a steep rise in the value of fish exported from Canada from about $\$83 \times 10^6$ to $\$123 \times 10^6$, and from 1951 on this value has fluctuated and was 148×10^6 in 1959. From Table VIII it will be seen that, with the exception of the very severe drop in herring oil and fish meal exports, there were no great differences in the 1956 and 1957 export values. In 1959, it has been stated that shipments were made to 83 different countries. The import value has always been very much lower and rather variable, the lowest figure ($\$5 \times 10^6$) was in 1950 and the highest $\$17 \times 10^6$) in 1956.

The fishery items exported from Canada are many and varied. Thus, in 1956 some 70 edible and 20 non-edible items were listed. These have been listed separately as regards quantities and values, and no adequate

data.,T4

group summaries appear to be available (Tables VIII and IX).

The countries to which exports are made are more numerous than the varieties of fish exported. Thus, 28 Commonwealth and 65 foreign countries, to which 19.7×10^6 and 114×10^6 worth of fish respectively were exported, are recorded in the 1958 report. The United States is by far the biggest customer, accounting for slightly over 98×10^6 in 1959. The remainder of the exports are largely to the United Kingdom (8.8×10^6), Jamaica (4×10^6), New Zealand, Cuba, Trinidad, Germany, Italy, Netherlands and Puerto Rico ($1 to $3 $\times 10^6$). Table X given a breakdown of the export values between 1955 and 1959 according to product classes.

Canadian imports of fish products amounted to about 17×10^6 in 1956 or roughly 12% of the export value. As with exports, many items were imported, chief among which were canned salmon, sardines, crab, shrimp and tuna.

In summary, it is very apparent that the United States is presently an ever-increasing importer of fish products, and Canada primarily an exporter. The United States takes over 70% of Canadian fishery products.

Composition and Nutritive Value

One has only to peruse the monumental work of Professor A. P. Vinogradov of Moscow on, "The elementary chemical composition of marine organisms" to appreciate the overwhelming amount of information which is available concerning this subject. Much of the data given in this book refer to marine invertebrates, and moreover to mineral composition. However, the water, nitrogen and ash composition of the soft portions of some 700 species of fish from all parts of the world is recorded, and also some "seasonal" analyses. Moreover, it is a valuable source of references. Other less comprehensive, but more recent articles, also give much information concerning chemical composition of fish.

When one considers this type of data it is immediately obvious that it is quite impossible to make any generalized statement about the elementary composition of fish unless a very wide latitude is allowed in presenting the data. Thus, the fat in herring changes seasonally from a few per cent to over 20 per cent, and the "gelatin" content of fish doubles just before spawning. It is indeed a well-established fact that maturation of fish is associated with a number of biochemical changes which often result in low fat content, muscular "wasting" with decrease in myosin, increase in proteins soluble in salt solutions of low ionic strength, and a probable increase in stroma proteins. In general, sexually mature fish are economically less desirable than well-fed less mature fish. For the present purpose it is assumed that fish are normally used when they are not at the height of sexual maturity.

From the nutritional standpoint fish are usually divided arbitrarily

into two groups, fatty and non-fatty. On the Pacific seaboard of the North American continent the most important commercial species are, with few exceptions, fatty fish such as salmon, tuna, herring and pilchards (sardines). These belong to the anadromous and pelagic classes of fish. Fatty fish traditionally make the best canned products, hence the widespread west coast fish-canning industry. The bottom fish (demersal) such as halibut, cod species, flounder and soles are usually of low fat content and may be used fresh, frozen or cured.

The "protein" content of a food may be quite high, but its nutritional value may be low unless it has the desirable content of all the "indispensable" (or "essential") amino acids. Moreover, these amino acids must be available to the animal. Amino acid analyses as determined by assays of chemically hydrolyzed proteins may be very misleading, for some of the essential amino acids may be "tied up" in such a way as to prevent their enzymic liberation in an animal's digestive tract. It is often necessary to carry out in vitro enzymic hydrolysis before assaying a protein for amino acids, or to determine deficiencies by time-consuming biological tests using animals. Cereal proteins are usually deficient in lysine, tryptophane, methionine or valine, and are of inferior nutritive value, especially if heated (toasted) at high temperatures. Fortification of wheat flour with a little lysine raises its protein nutritive value to the status of a good fairly complete protective protein. Fish muscle proteins are of very high nutritive value.

On the North American continent only the muscle portion of fish is consumed to any extent, though occasionally livers, milts and roes are utilized for human food. In parts of Asia the viscera, visceral organs and even whole fish are used, sometimes in the preparation of sauces and digests (autolysates). According to available data the protein content of marine fish and shellfish varies from about 9 to 26%, and of salted and dried fish from about 30 to 80%. These are extreme values. The muscle portion of fish is the only one for which comprehensive data on protein and amino acid composition are available, and this normally represents 40 to 60% of the total weight of fish, though exceptionally high values such as 60 to 70% have been reported for herring. There are also considerable variations in composition in different parts of the muscle tissue. Some general values for the elementary composition of some important Pacific edible fish are given in Table XI.

As mentioned previously, fish muscle is an excellent source of protein of high biological value. This is obvious from Table XII which gives the content of essential, and certain other, amino acids in the flesh of several common Pacific Ocean food fish in comparison with the mixed proteins of wheat flour and of whole egg. It will be noticed that in general fish flesh has a slight deficiency in arginine, tryptophane, methionine, threonine, leucine and isoleucine in comparison with whole egg protein, but is somewhat richer in histidine and lysine. When compared with fish flesh the wheat protein is very deficient in lysine, and also slightly deficient in methionine, arginine, histidine and threonine.

data.,T6

In addition to their excellent content of essential amino acids, fish muscle proteins have high coefficients of digestibility and, where measurements have been made, high protein efficiency ratios (1.5 to 3.0). The caloric value of fish is variable, and is of course much higher in the case of fatty fish. Thus, values (calories/100 g) of 75, 104 and 176 have been given for fish fillets which contain 0.2, 2.5 and 10% respectively of fat.

In general, fish flesh is a poor source of Vitamin A but the flesh of fatty fish is often a good source of Vitamin D. Fish muscle is a poor source of thiamin and riboflavin, but a fairly good source of pyridoxine, biotin and niacin. Fish livers, which are with many species extremely oily, where analytical data are available, have been found to contain protein of high nutritive value, and are rich in vitamins of the B complex and A and D. Fish eggs contain protein of high nutritive value. Fish milts are largely composed of nucleoprotamines, which are probably of poor nutritive value in themselves. Milts have been reported to be a good source of vitamin E.

In conclusion, there is abundant evidence in support of the fact that normal processing procedures such as freezing and storage, canning and dehydration, do not normally appreciably decrease the nutritive value of fishes, though some of the more labile vitamins of the "B complex" may be destroyed by canning or dehydration procedures.

Processing Methods and Uses

Table XIII indicates roughly the utilization pattern of fish in several countries which have large fisheries in the Pacific. It will be noted that Japan and Russia, with large rapidly-developing fisheries, cure (probably largely salting) about half their fish, while about 60% of the fish is consumed (or exported) fresh and frozen in the United States and Canada. The Japanese consume quantities of fish made into cooked products known as kamaboko, and also favour consumption of really fresh fish without any form of processing. These procedures are rare on the North American continent. There is little doubt that rising standards of living eventually tend to an increase in consumption of high quality fresh frozen and canned fish and decreases in that of salted products.

Table XIV summarises very generally the quantities and values of different classes of manufactured fish and shellfish products in the United States in 1957. Of the 232 million pounds of tuna canned that year, valued at 136×10^6, 34% was from imported fish. The canned salmon pack was 154×10^6 pounds valued at 86×10^6 to the canners, and that of Pacific and jack mackerel 60×10^6 pounds, valued at 7.4×10^6. Anchovies were scarce and the pack was only 13.8×10^6 pounds valued at 2.3×10^6. It is important to note that the value of canned fish products in the United States more than trebled from about 100×10^6 in 1939 to nearly 350×10^6 in 1947, but that in the following decade the production was almost static with a rough average of 325×10^6. This general trend also occurred with by-products. Most of these products are consumed domestically since exports of these commodities are small.

data.,T7

So far as Canada is concerned it is difficult to determine the exact pattern of domestic utilization of fish since the export figure is comparatively large. It has already been indicated (Table XIII) that canned plus fresh, frozen and cured fish each account roughly for one-third of the fish consumed in Canada annually. Since, in 1957 147×10^6 worth of fish products were exported and the landed value to fishermen was 94.5×10^6, it is evident that domestic consumption is not large; in fact, it was about 13 lb per capita in 1957.

What are the Prospects?

Since my own training and research interests have centred almost entirely in the fields of microbiology and biochemistry, generally as applied to foods, I am in no position to give any authentic predictions. The present outlook certainly is not particularly bright, for there are several threatening situations which could depress the North American fishing industry more or less severely. In general, these are occasioned by the ever-increasing fish production and studious application of almost all available technological principles by other large producers, particularly the Asiatic countries and the U.S.S.R. This could lead to decreased fisheries in certain presently uncontrolled areas with consequent increase in overall production costs in North America. Since our comparatively high production costs cannot be strained much further, we might be forced to resort to importing more cheaply produced fish from other fishing nations. This effect is being felt in several areas already.

One of the present points of worry is the United States domestic tuna industry about which so much has been said and published in recent years. In 1947 a little over 100×10^6 lb of canned tuna was consumed in the United States. This increased steadily and was 277×10^6 lb in 1957. Figures show that most of this increase has come from imported frozen or canned tuna; the domestic fishery is slowly decreasing. This is putting United States fishermen out of work. Much smaller, but locally just as vital problems are the British Columbia herring and whale meal industries, which ceased to function a few months ago. The reason for this is comparatively cheap imported meal from South America flooding the usual Canadian markets. One real danger spot, of course, is the Newfoundland fishery, where cheap landings of frozen cod in the traditional Canadian markets to the south could prove very damaging. In addition, as noted above, exploitation of traditional North American fishing grounds is occurring, and will almost certainly increase, and probably raise our production costs due to fish scarcities developing on the fishing grounds. Domestic production of valuable Pacific salmon species has been severely curtailed in some rivers by power dams, and the struggle between power and fish interests is still proceeding.

Are there any solutions to these problems? A miraculous raising of living standards in other countries so that prepared or other fishery products could be exported profitably to them is unlikely. Unfortunately

the countries with the largest consumer populations are either major fishing nations or are those with which we do not trade for political reasons. Now many of the Pacific Rim countries produce fruits and fruit products which are not indigenous to North America, and a useful mutual trade may continue to develop in this area. Fish products are unique sources of protective protein, while comparatively speaking, fruits are more favoured for quite other reasons. Perhaps the real solution lies at the door of the food technologist who, well trained in the disciplines of organic chemistry, biochemistry and microbiology may in the future produce desirably flavoured protein products from varieties of fish which normally have little food appeal.

Though the general outlook I have pictured is a little gloomy, there are a few bright spots on the horizon. Thus this year tuna landings in the San Pedro area have improved markedly due to the adoption of seining instead of the traditional pole fishing. The Japanese have been reported to be considering an embargo on Peruvian fish meal, so that we are not the only ones adversely affected by cheap production in that area. Perhaps in other fields, biological, oceanographic and technological findings may come to the aid of the fishing industry.

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TABLE I. Weight of fish landed (metric tons x 10^3)

Varieties	U.S.A. 1953	U.S.A. 1958	Canada 1953	Canada 1958	Japan 1953	Japan 1958	U.S.S.R. 1953	U.S.S.R. 1958	Mainland China 1953	Mainland China 1958	Philippines 1953	Philippines 1958
Freshwater fishes	73.4	68.0	36.6	43.3	25.0	40.7	473.2	460.2	630	1180‡		
Salmon, trouts, smelts, etc.	149.2	144.7	119.0	109.6	60.5	226.1	230.9	116.8				
Flounders, halibuts, soles, etc.	66.7	78.9	53.1	64.6	128.4	180.6	63.6	203.0				
Cods, hakes, haddocks, etc.	141.0	200.9	362.5	387.7	233.1	344.9	341.0	367.6				
Herrings, sardines, anchovies, etc.	910.8	957.5	250.3	291.8	935.2	672.0	670.3	1,093.1				
Tunas, bonitos mackerels, etc.	150.6	172.4	13.5	10.7	507.3	813.0	20.5	5.9				
Mullets, jacks, sea basses, etc.	194.9	237.0	26.5	33.7	1,022.0	1,409.1	48.8	291.2				
Sharks, rays, etc.	3.1	10.4	0.5	0.3	97.4	82.9	*	*				
Unsorted and unidentified fishes	54.4	*	1.8	4.0	260.9	308.9	99.8	40.3				
Crustaceans	198.1	183.7	23.1	22.2	74.4	119.3	29.1	31.7				
Molluscs	492.4	616.4	26.4	19.8	828.2	919.2	*	*				
Aquatic animals	2.9	1.4	*	*	26.8	48.6	5.8	11.2				
Aquatic plants	*	*	10.9	15.4	302.4	339.7	*	*				
TOTALS	2,437.5	2671.3	924.2	1,003.1***	4,521.6	5,505.0	1,983.0	2,621.0	1,900	6,000**	311.9	447.3

‡ 1957

* Not available or included in another figure

** This figure would seem open to serious question since in 1956 the figure was similar to the United States, namely, 2640 x 10^3 metric tons. However, there is every indication that the catch is increasing very rapidly.

*** The 1959 value was 1040

TABLE II

Approximate loadings of fish which probably originate in the Pacific Ocean (metric tons x 10^3)

		1953	1958
U.S.A.	Pacific	332.9	410.0
	Alaska	134.7	172.4
	Total	467.6	582.4
Canada	British Columbia	247.1	296.9
Japan	North Pacific		
	(salmon, factory ship)	14.7	91.6
	(crab fishery, " ")	2.4	20.8
	otter trawl		56.2
	Domestic marine fishes	4260	4951.4

TABLE III.

Value of fish landed (U.S. dollars x 10^3)

	U.S.A. (including Alaska)		Canada	
	1953	1957	1953	1958
Freshwater fishes	19,088	17,911	7,894	9,671
Salmon, trouts, smelts, etc.	41,101	40,851	29,149	43,985
Flounders, halibuts, soles, etc.	16,024	18,364	7,359	11,352
Cods, hakes, haddocks, etc.	16,317	18,262	17,336	19,786
Herring, sardines, anchovies, etc.	25,696	31,153	6,523	10,024
Tunas, bonitos, mackerels, etc.	49,768	40,624	2,207	2,324
Mullets, jacks, sea-basses, etc.	28,828	29,421	1,637	2,217
Sharks, rays, etc.	133	336	147	50
Unsorted and unidentified species	2,193	56	119	260
Crustaceans (shrimp, lobster, etc.)	98,560	98,497	16,502	16,517
Molluscs (clams, oysters, etc.)	53,813	53,948	2,418	2,271
Aquatic animals	*	1,116	*	*
Aquatic plants	*	*	319	449
‡ Totals	351,581	350,539	91,610	118,906

* Not available or included in another figure

‡ The totals for the following countries in 1953 and in 1958 were:
Australia, 17,889 and 23,489; Hawaii 3,798 and 2,632;
Japan 457,484 and 671,392; Chile 3,473 and 9,248;
New Zealand 5,438 and 7,392; Philippines 147 and 203;
China (Taiwan) 42.6 and 43.

TABLE IV.

Quantities and Values of Fish Products in the United States (1957)

Product	Quantity ($lb \times 10^6$)	Value ($\$ \times 10^6$)
Packaged raw, frozen and breaded fillets, steaks, fish sticks, etc.	480	266
Canned (tuna, salmon, shellfish etc.) for human consumption	644	301
Canned for animal foods and bait	348	35
Cured fish (salted, smoked, dried shrimp, etc.)	74	38
By-products: Meal, oils, solubles, shell grits, etc.		81
TOTAL		722

TABLE V

Exports of fishery products, U.S.A., 1957

Item	($lb \times 10^3$)	($\$ \times 10^3$)
Fresh and frozen fish and shellfish	15,539	3,901
Canned fish and shellfish	68,316	16,046
Cured fish and shellfish	1,067	447
Marine oils	117,301	10,993

TABLE VI

Imports of Fishery Products, U.S.A., 1957

Item (Edible)	(lb x 10^3)	($ x 10^3)
Fresh-water fish, fresh and frozen	38,320	11,134
Salt-water fish " " "	261,594	35,022
Fillets, steaks, blocks, etc.	203,978	49,142
Fish sticks	80	31
Shellfish, etc., fresh and frozen	638,018	170,999
Canned fish	128,527	46,428
Canned shellfish	147,371	61,345
Pickled, salted and all cured products	84,650	13,614
Other	13,985	3,998
Total edible imports	884,024	250,956
Total non-edible imports (shells, pearls, sponges, agar, fish solubles, meals, etc.)		46,487
Grand Total		297,443

TABLE VII. Imports of Fishery Products into the United States in 1957 by type of products

Item	(lb x 10^3)	($ x 10^3)
Fresh and frozen tuna	139,287	16,765
Shrimp	69,676	36,415
Ground fish fillets, steaks, etc.	140,678	27,417
Other " " " "	63,301	21,725
Canned sardines in oil	20,127	8,348
Canned tuna in oil	1,068	346
Canned tuna in brine	43,318	16,656
Canned bonito and yellowtail	15,513	3,663

TABLE VIII. Exports of certain of the more important fishery products from Canada, 1956 and 1957

Item	1956		1957	
	(lb x 10^3)	($ x 10^3)	(lb x 10^3)	($ x 10^3)
Dried salted cod	80,863	12,898	88,100	14,226
Fresh and frozen cod fillets	33,301	6,029	35,331	6,445
Fresh lobsters	24,249	16,845	24,587	16,413
Canned salmon	32,308	14,981	24,037	11,272
Fresh and frozen salmon	14,161	5,939	16,545	6,100
Whitefish, fresh and frozen	15,282	5,530	16,410	5,808
Flatfish, fresh and frozen	15,899	4,770	16,955	5,235
Haddock and other salted varieties	13,732	2,069	14,763	2,317
Halibut, fresh and frozen	15,416	4,594	18,556	5,330
Herring, fresh and frozen	26,141	409	45,923	505
Sardines, (etc.) in oil	11,025	2,117	11,522	2,276
Fish meal	107,920	7,594	86,707	6,185
Herring oil (gallons)	1,374,569	1,163	20,100	12.4
Total value of all exported fish products		133,707		132,454

TABLE IX. Value of exports of fisheries products from Canada 1955-59 ($ x 10^6)

	1955	1956	1957	1958	1959
Fresh and frozen	73.52	77.97	81.46	88.20	85.74
Cured	23.94	22.84	24.51	22.70	21.79
Canned fish and shellfish	20.20	19.63	16.02	35.63	28.00
Miscellaneous (meal, oil, etc.)	11.18	13.27	10.47	8.49	12.29
Total	128.84	133.71	132.46	155.02	147.82

TABLE X. Export values of fishery products to different countries by Canada, 1955-59 (4 x 10^6)

	1955	1956	1957	1958	1959
United States	21.98(71.4)	96.76	97.01	103.32	98.65(66.7)
British Caribbean	6.82(5.3)	7.39	7.93	6.85	7.91(5.4)
Non-British "	10.35(8.0)	10.09	9.76	9.67	9.03(6.1)
Europe	14.83(11.5)	15.26	13.29	31.50	28.75(19.4)
All other countries	4.87(3.8)	4.21	4.47	3.67	3.48(2.4)
Total	128.84(100)	133.71	132.42	155.02	147.82(100)

TABLE XI. Elementary composition of muscle of several species of commercially important Pacific Ocean fish

	Protein (% N x 6.25)	Fat	H_2O
Yellowfin tuna	24.7	-	71.5
" "	23.3	-	74.7
California bonito	24.0	-	67.6
Herring	19.5	-	72.5
* Sockeye salmon ♂	22.8 ± 0.29	5.07 ± 0.24	70.7 ± 0.23
* Sockeye salmon ♀	22.0 ± 0.67	5.59 ± 0.19	71.0 ± 0.12
White spring salmon	19.15	13.24	66.4
Pink salmon	20.5	6.0	71.0
Red spring salmon	20.0	13.0	65.0

* Entrance of Fraser river before active river migration

TABLE XII. Amino acids in muscle tissues of certain Pacific Ocean fish as compared with wheat protein and whole egg protein

Source	% Protein	g amino acid per 16 g of nitrogen (i.e. per 100 g protein)											
		Arg	His	Lys	Tyr	Try	Pal	Cys	Met	Thr	Leu	ILeu	Val
Mackerel	21.2	5.2 -5.4	5.6 -6.0	8.3 -8.7		1.0 -1.1	3.7 -3.9		2.8	4.5 -4.6	6.7 -8.1	4.7 -5.0	4.8 -5.6
Sardines	20.8	4.9 -6.0	4.6 -5.5	8.6 -9.0		0.9 -1.0	3.8 -4.2		2.7 -4.2	4.3 -4.6	6.5 -8.2	4.2 -5.2	4.8 -5.6
Tuna	25.8	5.2 -5.7	5.6 -6.0	8.3 -8.6		0.4 -1.0	3.4 -3.6		2.8 -3.0	4.7 -6.3	6.9 -7.2	4.4 -4.8	5.1 -5.5
Spring (King) salmon		5.5 -5.8	2.7	7.5 -8.4		0.9	3.4 -3.7	1.20	2.8 -3.0	4.0	6.8 -7.2	4.7 -5.4	5.3 -5.8
Herring		5.9	2.0	8.2		0.7	4.5		2.2		8.0	6.4	5.1
Cooked pressed herring dried at 40°F.	71.6	6.5	2.5	12.2	2.6	1.1	4.0		2.5	4.1	7.4	4.9	5.4
Whole egg protein		6.4 -9.7	2.1 -3.6	6.0 -7.8	4.3 -5.0	1.1 -1.6	5.4 -6.3	2.1 -2.4	3.0 -4.0	4.3 -5.3	9.2 -19.0	5.3 -8.0	4.4 -7.2
Whole wheat protein	10.3 -19.2	3.1 -5.2	1.2 -2.6	2.2 -2.9	0.6 -4.4	1.0 -1.4	3.8 -5.7	1.7 -2.4	1.0 -2.5	2.5 -3.3	6.1 -13.0	3.5 -7.0	3.6 -4.8

Fish lower as a rule in all but lysine and histidine.

TABLE XIII. Utilization pattern of fish in several countries with large Pacific Ocean fisheries

	Fresh	Frozen	Canned	Cured
World	53	9	10	28
Japan	33	8	10	50
United States	45	13	38	4
Canada	22	36	8	34
U.S.S.R.	22	19	8	51

TABLE XIV. Quantities and values of the important classes of manufactured fish products in the United States in 1957

Class of product	(lb x 10^6)	($ x 10^6)
Fresh and frozen:		
Fillets and steaks	154.5	46.8
Wholemeat for animal feed, etc.	2.1	0.29
Breaded:		
Fish sticks	53.1	23.5
Fillets, etc.	21.4	8.8
Shellfish:		
Not breaded	172.1	127.9
Breaded	59.6	44.7
Specialties (fish and shellfish)	17.4	14.4
Sub-total	480.3	266.4
Canned:		
Fish and shellfish for human consumption	644.0	300.7
For animal food	346.7	34.1
Salmon eggs for bait	0.91	0.92
Sub-total	991.6	335.8
Cured fish and shellfish:		
Salted	35.9	12.0
Smoked	35.3	25.3
Other	3.0	0.11
Sub-total	74.2	38.4
By-products, including meal, marine oils, solubles, liquid fish, shell products		81.4
Grand total:		722.0

THE REPRODUCTIVE PERFORMANCE OF BROAD BREASTED BRONZE TURKEYS MAINTAINED ON SLATTED FLOORS

BY

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Reprinted from POULTRY SCIENCE: Vol. XL, No. 1
January, 1961

FRB No. 651

The Reproductive Performance of Broad Breasted Bronze Turkeys Maintained on Slatted Floors

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(Received for publication May 2, 1960)

THE hatchability of turkey eggs, as a general rule, declines after the 9th to the 12th week of the breeding season (Dickens *et al.*, 1941; Atkinson *et al.*, 1955; Jensen *et al.*, 1956). This is particularly true of the hatchability of eggs from broad-breasted stock, and it has been suggested that the hens may become depleted of some nutrient or nutrients. In recent years the importance of vitamin E, niacin, pantothenic acid and folacin has been established in the maintenance of hatchability (Jensen, 1953; Scott and Nelson, 1955; Atkinson *et al.*, 1955; Kratzer *et al.*, 1955, 1956; Ferguson *et al.*, 1956).

Declining hatchability of fertile eggs is usually accompanied by a decline in fertility. Atkinson *et al.* (1955) reported that supplementation of diets with dehydrated alfalfa meal and condensed fish solubles resulted in maintaining fertility at a high level throughout a 17-week breeding period.

The object of the present experiment was to determine whether supplementing a supposedly adequate breeder ration with condensed herring solubles as a source of unidentified factors and with additional amounts of known vitamins would have any effect upon the reproductive performance of Broad Breasted Bronze turkeys during a 20-week breeding season.

EXPERIMENTAL

One-hundred and sixty Broad Breasted Bronze turkey hens which had been reared on wire and in pens with slatted floors at The University of British Columbia were distributed at random among 16 groups of 10 birds each. Forty Broad Breasted Bronze males of the same strain and reared under similar conditions were randomized among 4 groups of 10 birds each. To indicate the size and body conformation of the strain used the average body weights and breast widths of the birds are shown in Table 1. All the birds had been fed practical rations during the growing period.

The composition of the breeding rations is shown in Table 2. The level of vitamins in the basal ration met the requirements for turkey breeding stock (Kratzer, 1959). Each treatment was replicated 4 times, treatments being randomized to pens using the permutations of random numbers (Cochrane and Cox, 1957).

In December, 1958 each group of 10 hens was placed in a 12′×12′ pen with a slatted floor. Each pen was equipped with a 6′ feed hopper, a water trough and 4

TABLE 1.—*Mean body weight and conformation of turkeys at end of twenty week breeding season*

Diet	Sex	Body weight	Breast width	Keel length
1	F	16.1 lbs.	4.6 ins.	6.6 ins.
	M	29.7	5.7	8.7
2	F	16.3	4.6	6.5
	M	31.0	5.8	8.9
3	F	15.6	4.7	6.5
	M	29.5	5.8	8.4
4	F	16.5	4.7	6.5
	M	32.7	6.1	8.8

t boxes. Feed and water were supplied *libitum*. The males were lighted (on uary 19, 1959) 3 weeks before the fe-les, with an increasing amount of light m 10 hours per day during the first k to provide a minimum of 13 hours ight per day during the third and sub-uent weeks. Individual pen matings e made with males which had been the same rations as the females with

TABLE 2.—*Composition of rations*

Ingredient	Per 100 lb.
on 1.—Basal ration.	
Wheat	59.0
Oats	10.0
Barley	10.0
Dehydrated cereal grass, 20% protein	2.0
Soybean oil meal, 44% protein	10.0
Meat meal, 50% protein	2.0
Limestone	3.5
Bone meal	2.5
Iodized salt	0.5
Manganese sulphate	0.02
Feeding oil (2,250 I.U. vitamin A gm.)	0.5 gm.
Riboflavin	0.18 gm.
Niacin	1.5 gm.
Calcium pantothenate	0.8 gm.
α-tocopheryl acetate	0.25 gm.
Vitamin B_{12}	0.0001 gm.
on 2.—Basal ration plus 3% condensed herring solubles.	
on 3.—Basal ration plus 3% condensed herring solubles plus the following vitamins per pound	
Vitamin A	8,000 I.U.
Vitamin D	1,200 I.C.U
α-tocopheryl acetate	38.0 mg.
Riboflavin	3.6 mg.
Calcium pantothenate	16.0 mg.
Niacin	30.0 mg.
Folacin	0.8 mg.
Choline chloride	1.8 gm.
Vitamin B_{12}	2.0 mg.
on 4.—Basal ration plus 3% condensed herring solubles plus 0.8 mg. folacin per pound	

TABLE 3.—*Egg production, mortality and replacement*

Egg production and mortality in hens

Ration	Egg production egg/hen housed	No. of hens dying
1	59.6	2
2	50.6	4
3	55.1	1
4	51.8	7

Males replaced in breeding pens

Ration	Replaced	Reason for replacement
1	May 19	Thin, poor condition
2	April 2	Died
3	—	—
4	July 6	Thin, poor condition
	July 6	Thin, poor condition

which they were to mate. Although 10 males were maintained on each experimental ration the original males put into the breeding pens were used except for the replacements indicated in Table 3. The males were rotated weekly within treatments. Egg production and mortality in the hens is given in Table 3.

The eggs were gathered 4 times daily, marked with the pen number and date, and were stored at 55–60°F. The first setting of eggs was made on March 18, 1959. Settings were made once weekly. All eggs were individually candled at 7, 14, and 24 days of incubation. All clear eggs and eggs containing dead embryos were removed and broken open to determine the approximate time of embryonic death.

RESULTS AND DISCUSSION

The results on fertility, embryonic mortality and hatchability by 4-week periods and for the entire experiment are shown in Table 4.

It will be seen that fertility was high throughout the whole of the experimental period (average 89.9%). There was a greater drop in fertility in the basal group (1) and in the group (2) fed the supple-

TABLE 4.—*Summary of incubation results*

Week	Ration	No. of eggs set	Percent infertile	Embryonic mortality percent of fertile eggs			Percent hatchability (fertile eggs)	Percent hatchability (all eggs)
				0–7 days	8–24 days	Dead-in-shell		
1–4	1	645	10.5	3.5	3.3	10.4	82.8	74.1
	2	580	6.9	6.3	4.8	11.5	77.4	72.1
	3	641	7.5	7.9	4.0	12.1	75.9	70.2
	4	606	12.7	4.2	4.9	14.6	76.4	66.7
	Total	2,472	9.4	5.5	4.2	12.1	78.2	70.8
5–8	1	675	6.4	7.1	1.4	13.9	77.5	72.6
	2	531	7.3	7.3	1.4	13.4	77.8	72.1
	3	534	13.1	13.2	1.1	9.3	76.3	66.3
	4	473	10.4	7.3	2.4	13.9	76.4	68.5
	Total	2,213	9.1	8.6	1.5	12.7	77.1	70.1
9–12	1	467	5.8	5.2	5.7	11.6	77.5	73.0
	2	469	7.9	13.2	3.9	13.4	69.2	63.8
	3	482	6.4	7.8	3.6	15.7	72.7	68.0
	4	433	10.2	8.0	6.9	11.0	73.5	66.1
	Total	1,851	7.5	8.5	5.0	13.0	73.5	67.7
13–16	1	311	12.9	9.6	5.5	16.2	68.6	59.8
	2	212	6.1	9.0	7.0	24.1	59.3	55.7
	3	252	6.7	11.9	5.5	19.5	63.1	58.9
	4	312	17.3	11.2	5.8	19.4	63.6	52.6
	Total	1,087	11.4	10.5	5.9	19.5	64.0	56.8
17–20	1	170	30.0	7.6	8.4	20.2	63.9	44.7
	2	96	8.3	20.4	10.2	27.3	42.0	38.5
	3	154	18.8	13.6	4.8	24.0	57.6	46.8
	4	115	33.9	10.5	6.6	15.8	67.1	44.3
	Total	535	23.7	12.8	7.4	22.1	57.8	44.1
0–20	1	2,268	10.1	6.0	3.8	13.1	77.0	69.3
	2	1,888	7.3	9.3	4.2	14.7	71.7	66.5
	3	2,063	9.5	10.1	3.4	14.0	72.4	65.6
	4	1,939	13.6	7.2	5.0	14.4	73.3	63.4
	Total	8,158	10.1	8.1	4.1	14.0	73.7	66.2

ment of condensed herring solubles plus folic acid than in the other two groups after the 16th week. Since only 4 males are represented in each dietary treatment, however, the differences in fertility can hardly be ascribed to the diet fed.

Egg production was similar for hens on all diets and the rate of lay declined at the same rate in each treatment.

Hatchability of fertile eggs during the first 12 weeks of the experiment for groups 1 to 4 respectively was 79.4, 75.1, 75.1, and 75.6%. During the last 8 weeks of the experiment hatchability of fertile eggs from the respective groups was 67.2, 54.0, 61.2 and 64.4%. Embryonic mortality during the 1st, 2nd–3rd and 4th week of incubation in each treatment throughout the experiment is shown graphically in Figure 1.

As would be expected, two peaks of embryonic mortality occurred, viz. during

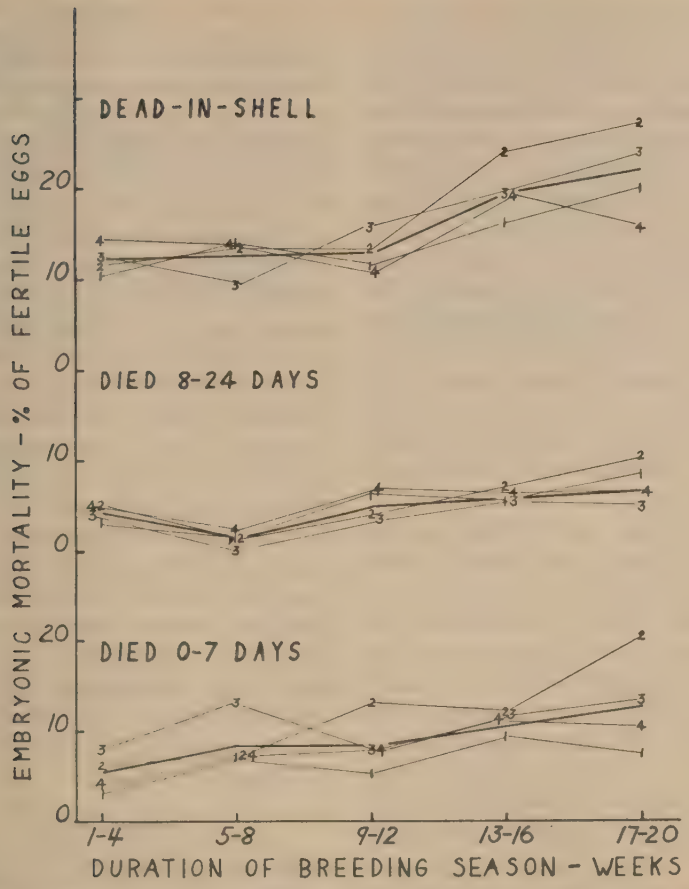

FIG. 1. Turkey embryonic mortality over a 20-week breeding season with the following vitamin supple nts to the basal ration: 1. basal ration; 2. 3% condensed herring solubles; 3. 3% condensed herring sol es plus additional pure vitamins; 4. 3% condensed herring solubles plus folacin. (The heavy line is the rage mortality for all treatments.)

e first 7 days and during the last 4 days incubation. The decrease in hatchabil- which occurred as the season advanced s principally due to an increase in bryonic mortality during these 2 pe- ds. It is interesting to note, however, at there was a general increase in mor- ity throughout the season at all stages embryonic development.

The reason for the failure of condensed n solubles to improve hatchability over prolonged breeding season in the present experiment as compared with the favorable response obtained by Feldman *et al.* (1957) no doubt lies in the composition of the basal diets employed in the respective experiments. The diet used in the present experiment contained wheat, oats, barley soybean oil meal, meat meal and dehydrated cereal grass. The diet used by Feldman *et al.* was made up of corn, milo and soybean oil meal in addition to minerals and pure vitamins. With our basal diet the hatchability of fertile eggs during

the 13th to the 20th week of the experiment was 67.2%. Since supplementing this diet with condensed herring solubles and additional pure vitamins was without effect on egg production or hatchability it is apparent that the decline in egg production and hatchability did not occur because the hens became depleted of vitamins as the season progressed. The data do not invalidate the conclusion from the results of a previous experiment that condensed herring solubles is an effective vitamin supplement (March *et al.*, 1959) since in the present experiment the basal diet used was already adequate for breeding stock without additional vitamin supplementation.

Of interest are the following percentages of eggs which were classed as dead-in-shell but which did pip: basal ration, 40.0%; basal ration plus condensed herring solubles, 36.8%; basal ration plus condensed herring solubles plus vitamin, 38.5%; basal ration plus condensed herring solubles plus folic acid, 41.9%. The similarity in these figures suggests that the rations were adequate and that perhaps incubation conditions were not optimum.

It is concluded from the results obtained that the vitamin levels recommended by Kratzer (1959) are sufficient to maintain fertility and hatchability in Broad Breasted Bronze turkeys over a prolonged breeding season. Condensed herring solubles, although a good source of the vitamin B-complex, evidently did not supply any unidentified hatchability factors not already present in the components of the basal diet. The decline in general reproductive capacity that had taken place by the end of 20 weeks could not be ascribed to nutritional deficiencies. Physiological factors inherent in the breed and strain of birds used in the test appear to have been responsible.

SUMMARY

Hatchability of turkey eggs over a 20-week period was studied to determine if the decline in hatchability were due to depletion of vitamins in hens after production. A total of 8,158 eggs was set from hens fed 4 different diets. The hens had been reared on wire and were kept on slatted floors during the growing and breeding season. Males were rotated among the 4 pens of hens fed each diet.

The basal diet supplied the vitamin levels recommended by Kratzer (1959). With this diet, no improvement in egg production, fertility or hatchability was observed in response to supplements of 3% condensed herring solubles (ration 2), 3% condensed herring solubles plus additional pure vitamins (ration 3) and 3% condensed herring solubles plus folacin. Over the 20-week period fertility was 89.9% and hatchability was 73.7% of fertile eggs. Hatchability of fertile eggs was 76.4% during the first 12 weeks of the season and 62.2% in the period from 12–20 weeks.

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APPLICATION OF THE VARRENTRAPP REACTION TO THE PREPARATION OF LONG-CHAIN DICARBOXYLIC ACIDS

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F.R.B. No. 652

APPLICATION OF THE VARRENTRAPP REACTION TO THE PREPARATION OF LONG-CHAIN DICARBOXYLIC ACIDS

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Aliphatic dicarboxylic acids with 11 or more carbon atoms were required for gas–liquid chromatographic identification of similar materials produced by ozonolysis of marine-oil fatty acids. Aside from brassylic acid, prepared by oxidative fission of erucic acid, these acids are not conveniently available. As an alternative to individual syntheses, modification of the Varrentrapp reaction (1–4) by limiting it to redistribution of the 13:14 double bond in erucic acid into adjacent positions, followed by oxidative fission and recovery of the dicarboxylic acids, offered a means of obtaining a number of these acids in one preparation.

The product of alkali fusion at 320° C was selected for oxidative fission as the iodine value of 60 indicated that the Varrentrapp reaction proper, production of the saturated acid two carbon atoms shorter than the original monounsaturated acid, had proceeded only to a limited extent. Since double bonds migrating to the 6:7 or 7:8 positions rapidly participate in the final stages of this reaction (2), it was expected that the residual double bonds in the fusion product would be in positions more remote from the carboxyl group.

The petroleum-ether-soluble ozonolysis product contained chiefly monocarboxylic acids ich as palmitic, stearic, and arachidic, with appreciable amounts of odd-numbered onocarboxylic acids of 11 or fewer carbon atoms. The methanol-soluble ozonolysis roduct contained approximately 60% of roughly equal amounts of the dicarboxylic ids with 7 to 16 and 17 carbon atoms and 40% of monocarboxylic acids with 7 to 16 rbon atoms (Fig. 1).

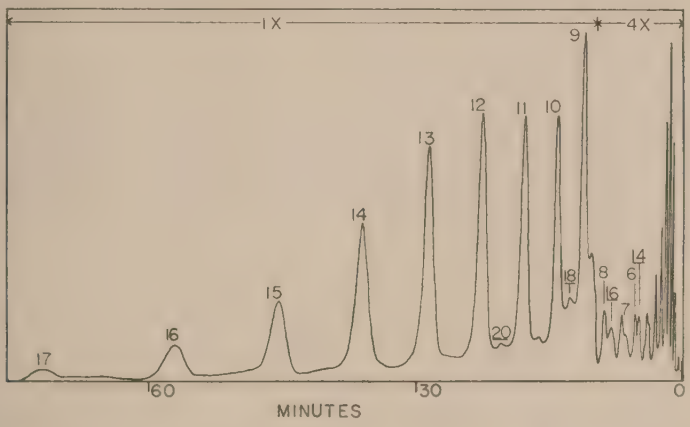

FIG. 1. Gas-liquid chromatogram of methyl esters of dicarboxylic acids. Column, 10 ft × ¼ in., packed ith 25% LAC-3R-728 on 60-80 mesh Chromosorb. Analysis of 0.005 ml at 200° C with hydrogen carrier as flowing at 80 ml/minute. Numerals indicate the number of carbon atoms in the acid chains, mono-rboxylic acids being underlined.

Since double bonds are increasingly less susceptible to the Varrentrapp reaction when ore remote from the carboxyl group, a higher proportion of long-chain dicarboxylic cids with 15 or more carbon atoms could be obtained by prolonged fusion at lower emperatures. The over-all dicarboxylic acid yield would, however, be diminished as a onsequence of further completion of the Varrentrapp reaction.

EXPERIMENTAL

A technical erucic acid, iodine value 85, was employed in this reaction. Gas–liquid hromatography indicated a composition of 72.8% erucic acid, 12.4% eicosenoic acid, .7% oleic acid, and minor amounts of palmitic, stearic, linoleic, and arachidic acids. lkali fusions were carried out in a covered iron vessel fitted with a nitrogen inlet and stirrer of iron pipe. Equal weights of erucic acid, sodium hydroxide, and potassium ydroxide were placed in this vessel, and it was then placed in a Wood's metal bath reheated 40° C above the reaction temperature. The mixture was stirred continuously or ½ hour and intermittently for a further half hour. The cooled reaction mass was issolved in water, acidified, and the fatty acids extracted with petroleum ether. Weight ecoveries were approximately 80%, with iodine values of 38, 60, and 69 from fusions at, espectively, 360, 320, and 300° C for 1 hour. Gas–liquid chromatography indicated almost omplete destruction of the oleic, linoleic, and eicosenoic acids, the products thus con-sting chiefly of saturated acids and docosenoic acids.

The alkali fusion product of iodine value 60 (5 g) was ozonized in glacial acetic acid nd the ozonide decomposed with hydrogen peroxide (2). The final solution was diluted

to 400 ml with water and extracted with diethyl ether (400 ml). The ether solution was washed with water (50 ml), and the aqueous phases discarded. The ether was removed and the product (5.7 g) was dissolved in 90% methanol (200 ml) and extracted with petroleum ether (b. p. 40–60° C, 200 ml). The petroleum-ether-soluble material (0.9 g) and the methanol-soluble material (4.8 g) were converted to methyl esters with diazomethane. Identification of certain components was effected through mixed gas–liquid chromatograms with authentic materials employing both silicone grease and polyester substrates. The identification of the remainder was confirmed by plots of logarithm retention time vs. number of carbon atoms.

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Received April 21, 1961.
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α-REDUCTION OF THE 20-CARBONYL GROUP IN C-21 STEROIDS BY RHODOTORULA LONGISSIMA[1]

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Abstract

The microbiological synthesis of several 20α-dihydro reference steroids is described. The influence of various substituent groups on α-reduction of the C-20 carbonyl group by a yeast, *Rhodotorula longissima*, has been investigated for compounds related to progesterone and corticosterone. Reduction at C-20 is enhanced by the presence of a 17α-hydroxyl group, but retarded by the presence of hydroxyl groups at C-11 and C-21. In those instances where 20α-conversion proceeds at a slow rate, oxygenation at C-11 appears to be essential for the preservation of the Δ^4-3-ketone structure.

Introduction

With the demonstration that reduction of the carbonyl at C-20 is a major pathway in corticosteroid metabolism (1–5), and especially since the isolation of 17α,20β-dihydroxy-Δ^4-pregnen-3-one from the plasma of postspawned female sockeye salmon (6), particular attention has been directed in this laboratory to the C-20-hydroxyl analogues of several adrenocortical steroid hormones.

The various enzymic transformations of the steroid molecule by microorganisms has been the subject of several reviews (7–9). In many cases the direct one-step microbial transformation has proved to be a tool equal to or superior to conventional chemical approaches which often entail many difficult steps and result in poor yields. One such case in point is the preparation of 20α-hydroxysteroids. The reduction of the C-20 ketone by various chemical methods yields the β-oriented 20-hydroxy compound as the major product (10–12). Recently, microbiological stereospecific conversions of Reichstein's substance S, cortisone, and prednisone to the corresponding 20α-hydroxysteroids were reported (13). A similar reduction of 17α-hydroxyprogesterone, in this laboratory, produced the 20α-dihydro-epimer in surprisingly high yield (6). It seemed desirable, therefore, to apply this facile method to the preparation of 20α-hydroxy epimers of various known adrenocorticoids. This paper is a report of the effects of common substituent groups in the steroid molecule on the rate of C-20 reduction and retention of the Δ^4-3-ketone entity.*

[1]Manuscript received February 9, 1961.

Contribution from the Fisheries Research Board of Canada, Chemistry Section of the Technological Station, Vancouver 8, British Columbia.

*A side reaction which occurs during incubation involves the reduction of the 3-carbonyl group (13) with subsequent loss of ultraviolet light absorbency. Therefore, ring A stability, as measured by ultraviolet light absorption, will be used in this paper to denote the preservation of the Δ^4-3-ketone structure.

Methods

The yeast used throughout this investigation was *Rhodotorula longissima*, strain Schering OFV No. 2.* The reactions were carried out in the Edamin–dextrose–cornsteep liquor medium described by Carvajal *et al.* (13). Freshly grown 2-day-old cells were used to inoculate the reaction medium and incubation was at 25° C on a rotary shaker.

The rate of steroid conversion was followed readily by a quantitative paper-chromatogram analysis of aliquots at various intervals. Periodic samples were removed from the incubation mixture and extracted three times with 2.5 volumes of distilled methylene chloride. In a typical separation of the reaction products, an aliquot of the methylene chloride extract containing 50–100 μg steroid was transferred to a titerlenmeyer and evaporated to dryness at 59° C under a stream of nitrogen. The walls of the flask were rinsed into the well with methylene chloride and again evaporated under nitrogen. The residue, dissolved in a small amount of methylene chloride:methanol (1:1) was applied to a 1-cm strip of specially washed Whatman No. 1 filter paper (6). In all cases the papers were equilibrated overnight and developed in a descending solvent system at 20° C. Each paper was dried in air and scanned with ultraviolet light. An area containing ultraviolet-absorbing material running at the same rate as reference substrate material was cut out as a strip and eluted with methanol or methylene chloride:methanol (1:1) by descending chromatography. A second ultraviolet-absorbing area containing the more polar 20α-reduction product was located and a strip of the same size as that containing the original material was cut out and eluted as before. The eluated steroids were read in methanol against appropriate paper blanks in a Beckman DU spectrophotometer at a wavelength of 240 mμ. The relative concentrations of the eluates were calculated from the optical density readings based on the molar extinction value of cortisol ($\epsilon = 15{,}900$).

A 4% ethanolic phosphomolybdic acid reagent was used to detect ring A reduction. The spray revealed the presence of a still more polar compound (blue spot) which was not visible under the ultraviolet lamp.

The infrared spectra of many of the 20α- and 20β-epimers have been determined.

Experimental

Progesterone (obtained from the Schering Research Division) (50 mg) was dissolved in 3 ml ethanol and added to 125 ml of inoculated medium. Aliquots were removed after 1, 3, and 7 days. The products were separated by paper chromatography in benzene:hexane (1:2) – 80% methanol solvent system developed for 2 hours. The steroids were eluted from the paper strips with methylene chloride:methanol (1:1).

11-β-Hydroxyprogesterone (obtained from Elite Chemical Co.) (19.7 mg) was dissolved in 2 ml of ethanol and added to 45 ml of inoculated medium. Aliquots

*Obtained from the culture collection of the Northern Regional Research Laboratories (USDA) at Peoria, Ill., as *Rhodotorula longissima* NRRL No. Y2343.

were removed after 2, 7, 15, and 21 days. The products were separated in the heptane:benzene (1:1) – 70% methanol solvent system developed for 3½ hours. The steroids were eluted with methanol.

11α-Hydroxyprogesterone (obtained from Elite Chemical Co.) (24.4 mg) was added to 47.8 ml of inoculated medium with 1 ml ethanol. Aliquots were removed after 2, 7, and 14 days. The reaction products were separated in the toluene – 70% methanol system developed for 2½ hours.

17α-Hydroxyprogesterone (obtained from Schering Research Division) (50 mg) was added to 100 ml of inoculated medium. Aliquots were removed after 1, 2, and 5 days. Separation of products was achieved in the heptane: benzene (1:1) – 70% methanol solvent system in 2½ hours. Steroids were eluted from the paper with methylene chloride:methanol (1:1).

11-Ketoprogesterone (obtained from Elite Chemical Co.) (27.5 mg) was added in 1 ml ethanol to 47.8 ml of inoculated medium. Aliquots were removed after 2, 4, 7, and 14 days. The steroids were separated in the toluene – 70% methanol during a 2½-hour run. The steroids were eluted from the paper with methanol.

11-Keto-17α-hydroxyprogesterone (obtained from Sharp, Merck and Dohme Research Laboratories) (10 mg) was dissolved in 1 ml ethanol and added to 35 ml of inoculated medium. Samples were removed in 2 and 5 days and chromatographed in the toluene – 70% methanol solvent system for 2½ hours. Compounds were eluted with methanol.

11β,17α-Dihydroxyprogesterone (obtained from Sharp, Merck and Dohme Research Laboratories) (5 mg) in 0.5 ml ethanol was added to 17 ml of inoculated medium. Aliquots were taken after 2 and 5 days and chromatographed in the toluene – 70% methanol system for 2½ hours. Elution was carried out with methanol.

17β-Hydroxy-17α(iso)-progesterone (obtained from Elite Chemical Co. (26.4 mg) in 1 ml ethanol was added to 44.8 ml of inoculated medium. Samples were removed after 1, 2, 7, 14, and 21 days. Chromatography was carried out with the toluene – 70% methanol solvent mixture for 2½ hours.

Corticosterone (obtained from Mann Research Laboratories) (48 mg) was dissolved in 3 ml of ethanol and added to 100 ml of inoculated medium. Aliquots were taken after 13 and 32 days and chromatographed in toluene – 70% methanol for 2½ hours.

17α-Hydroxycorticosterone (hydrocortisone; obtained from Sigma Chemical Co.) (50 mg) was added to 100 ml of inoculated medium with 3 ml ethanol. Aliquots were removed after 13 and 32 days and chromatographed in the toluene – 70% methanol solvent system for 16 hours. Compounds were eluted with methanol.

11α-Hydrocortisone (obtained from Elite Chemical Co.) (19 mg) was added with 1 ml of ethanol to 47 ml of inoculated medium. Aliquots were taken at 2, 7, 15, 21, and 28 days and chromatographed in benzene:chloroform (1:1) – 70% methanol for 5 hours. Steroids were eluted from the paper with methanol.

11-Dehydro-17α-hydroxycorticosterone (cortisone; obtained from Mann Research Laboratories) (50 mg) was dissolved in 3 ml of ethanol and added to 100 ml of inoculated medium. Samples were taken at 13 and 32 days. Chromatography was carried out in toluene – 70% methanol for 5 hours. Steroids were eluted with methanol.

11-Desoxycorticosterone (obtained from Sigma Chemical Co.) (50 mg) was dissolved in 3 ml of ethanol and added to 90 ml of inoculated medium. Aliquots were taken after 1, 3, 7, 14, and 28 days. Chromatography was performed in the toluene – 70% methanol system for 3 hours.

11-Desoxy-17α-hydroxycorticosterone (Reichstein's substance S; obtained from Sigma Chemical Co.) (50 mg) was dissolved in 3 ml of ethanol which was added to 125 ml of inoculated medium. Aliquots were removed after 1, 3, and 7 days. The products were separated in the toluene – 70% methanol solvent system in $3\frac{1}{2}$ hours. The steroids were eluted with methanol.

Results and Discussion

Sodium borohydride was employed to prepare several 20-dihydro-epimeric pairs of steroids (5, 6) which were then separated by paper chromatography (Table I). The microbiological reduction products of cortisone, Reichstein's

TABLE I

Paper chromatography of 20-dihydrosteroids

Compound	Solvent system	R_T (hours)*
11β,17α,20α,21-Tetrahydroxy-Δ⁴-pregnen-3-one (1)†	Toluene – 70% MeOH	.274
11β,17α,20β,21-Tetrahydroxy-Δ⁴-pregnen-3-one (2)	Toluene – 70% MeOH	.342
17α,20α,21-Trihydroxy-Δ⁴-pregnen-3,11-dione (3)	Toluene – 70% MeOH	.930‡
17α,20β,21-Trihydroxy-Δ⁴-pregnen-3,11-dione (4)	Toluene – 70% MeOH	.779‡
11β,20α,21-Trihydroxy-Δ⁴-pregnen-3-one (5)	Toluene – 70% MeOH	.908
11β,20β,21-Trihydroxy-Δ⁴-pregnen-3-one (6)	Toluene – 70% MeOH	1.21
17β,20α,21-Trihydroxy-Δ⁴-pregnen-3-one (7)	Toluene – 70% MeOH	2.28
11β,20β,21-Trihydroxy-Δ⁴-pregnen-3-one (8)	Toluene – 70% MeOH	3.93
17α,20α-Dihydroxy-Δ⁴-pregnen-3-one (9)	Hexane – propylene glycol (30% MeOH)	.136
17α,20β-Dihydroxy-Δ⁴-pregnen-3-one (10)		.216
20α-Hydroxy-Δ⁴-pregnen-3-one (11)	Heptane – 80% MeOH	.512
20β-Hydroxy-Δ⁴-pregnen-3-one (12)	—	

*R_T = rate of migration in cm/hour. Intended primarily to show the relative mobility of epimeric pairs.
†(1, 2) 20-dihydrocortisols; (3, 4) 20-dihydrocortisones; (5, 6) 20-dihydrocorticosterones; (7, 8) 20-dihydro-11-desoxycortisols; (9, 10) 20-dihydro-17α-hydroxyprogesterones; (11, 12) 20-dihydro-progesterones.
‡Note that the α-epimer is the least polar. The α-dihydro-epimer was more polar than the β for all other epimeric pairs investigated.

substance S, and 17α-hydroxyprogesterone had the same chromatographic mobilities as the corresponding α-epimers prepared chemically. Since each of these 20α-dihydrosteroids was identified, they were not investigated further (6, 13). In order to extend the finding that the Δ⁴-3-ketonic steroid formed by the microorganism was always the 20α-dihydro-epimer, additional reduction products were identified. The microbiological reduction products of progesterone, 11-keto-17α-hydroxyprogesterone and 11β,17α-dihydroxyproges-

terone, were chromatographed in the heptane – 80% methanol, heptane: benzene (1:1) – 70% methanol, and toluene – 70% methanol solvent systems, respectively. The corresponding 20α- and 20β-dihydro-epimers, prepared by borohydride reduction, were chromatographed at the same time. The relative mobilities (α/β) of the respective epimeric pairs were .62, .81, and .75. The mobilities of the microbiological reduction products were identical with those of the α-epimers prepared chemically. After 2 hours at room temperature, the microbiologically produced 20α-dihydro-epimers of progesterone, 11-keto-17α-hydroxyprogesterone, and 11β,17α-dihydroxyprogesterone gave sulphuric acid chromogens with maxima at 292 and 400 mμ (very weak), 282 and 392 mμ, and 283 and 393 mμ, respectively. The sulphuric acid chromogens produced by the chemically prepared α-dihydro-epimers were identical with those obtained for the corresponding microbiological reduction products.

The characteristic of substrate specificity has been observed in steroid transformation reactions carried out by microbial enzymes (7). From the results contained in Tables II and III it is clear that the course of 20-keto reduction by *Rhodotorula longissima* is considerably altered by minor changes in the functional groups attached to the steroid molecule.

This may be elucidated by an examination of the data obtained for progesterone and its analogues.* After an incubation period of 7 days only 18% of the ultraviolet-light-absorbing material remained for progesterone. Of this amount, 30% was the 20α-hydroxy reduction product. For the same incubation period with 11β-hydroxyprogesterone, 31.8% of the original ultraviolet absorption was recovered of which there was 5.5% of the 20α-reduction product. On the other hand, incubation of 11-ketoprogesterone resulted in a recovery of 46.4% of the ultraviolet-absorbing material added to the incubation mixture. Of this amount 57.8% was the 20α-conversion product. In view of the difference in the amount of ultraviolet-light-absorbing material remaining, it would appear that 11-oxygenation was important in the preservation of the Δ^4-3-ketone structure. Moreover, the presence of a carbonyl group at C-11 appears to enhance 20-keto reduction, since 20α-reduced 11-ketoprogesterone was found in the reaction mixture as early as the second day. In contrast, although the hydroxyl group at C-11 seemed to increase ring A stability, a slower conversion rate to the 20α-reduction compound was obtained for 11β-hydroxyprogesterone. It follows then that addition of an 11β-hydroxyl group inhibits 20-keto reduction.

With the addition of a 17α-hydroxyl group to the progesterone molecule, a marked increase was noted in the rate of 20α-reduction. The conversion of 17α-hydroxyprogesterone to 17α,20α-dihydroxy-Δ^4-pregnen-3-one appeared to be complete in 24 hours. Further evidence of the hindering nature of a hydroxyl group at C-11 may be seen in the much slower reaction rate of

*For convenience in reference, those compounds which do not possess the hydroxyl at C-21 will be described as belonging to the "progesterone" series, and those compounds with the C-21-hydroxyl group as belonging to the "corticosterone" series.

TABLE II

Progesterone series

Ultraviolet-absorbing material remaining and 20α-reduction product formed after incubation with *Rhodotorula longissima* (%)

Incubation period (days)		P*	11β-OH-P	11α-OH-P	17α-OH-P	11-Keto-P†	11-Keto-17αOH-P	11β,17α-Di-OH-P	17β-OH-17α(iso)-P
1	U.V.	35.2			79.6				96.0
	20α	<5			94.2				None detected
2	U.V.		71.8	61.7	79.2	71.6	100	72.0	86.9
	20α		5.1	—	94.2	40.9	100	17.7	None detected
3	U.V.	24.3							
	20α	<5							
4	U.V.					67.6			
	20α					45.3			
5	U.V.				55.2		96.6	86.6	
	20α				96.8		100	46.0	
7	U.V.	18.4	31.8	10.5		46.4			72.8
	20α	30.0	5.5	+		57.8			None detected
14	U.V.			2.91		19.0			42.2
	20α			+		100			None detected
15	U.V.		9.6						
	20α		18.5						
21	U.V.		9.9						35.7
	20α		19.1						None detected

*P = progesterone.

†The Δ^4-3-ketonic reduction product was not proved to be 20α-hydroxy-11-ketoprogesterone.

TABLE III

Corticosterone series

Ultraviolet-absorbing material remaining and 20α-reduction product formed after incubation with *Rhodotorula longissima* (%)

Incubation period (days)		C*	17α-OH-C (cortisol)	11α-Hydro-cortisone	11-Dehydro-17α-OH-C (cortisone)	11-Desoxy-C	11-Desoxy-17α-OH-C (substance S)
1	U.V.					42.5	41.5
	20α					None detected	40.0
2	U.V.			77.8			
	20α			None detected			
3	U.V.					50.4	28.7
	20α					None detected	95.0
4	U.V.						
	20α						
5	U.V.						
	20α						
7	U.V.			73.3		46.1	17.4
	20α			None detected		None detected	100
13	U.V.	68.0	88.0		91.0		
	20α	None detected	None detected		50.6		
14	U.V.					16.9	
	20α					None detected	
15	U.V.			55.2			
	20α			None detected			
21	U.V.			48.9			
	20α			None detected			
28	U.V.			40.9		7.4	
	20α			13.8		None detected	
32	U.V.	13.3	66.7		72.4		
	20α	None detected	18.8		95.4		

*C = corticosterone.

11β,17α-dihydroxyprogesterone where only 46% of the remaining steroid was 11β,17α,20α-trihydroxy-Δ^4-pregnen-3-one after a reaction period of 5 days. On the other hand, 100% conversion of 11-keto-17α-hydroxyprogesterone was effected in 2 days and the product was recovered in nearly quantitative yield even after 5 days' incubation.

The corresponding compounds of the corticosterone series (i.e. C-21-hydroxysteroids) were also subjected to reduction by this method in order to determine if the results obtained with compounds of the progesterone series had more general application. In agreement with the expectations based on the findings with progesterone and its analogues a good recovery of ultraviolet-absorbing material was found with cortisone and hydrocortisone even on prolonged incubation (32 days). Again, the 11-keto compound, cortisone, was more readily converted to the 20α-dihydro compound than was its 11β-hydroxy counterpart, hydrocortisone. It is worthy to note that a change in the hydroxyl group at C-11 from the normal β in hydrocortisone to an α-configuration decreased ring A stability on prolonged incubation. This phenomenon was observed with 11α-hydroxyprogesterone as well.

As a rule, the steroids of the C-21-hydroxy series possessed a greater degree of ring A stability than did the 21-desoxy analogues, but reduction of the C-20 carbonyl proceeded at a slower rate. For example, the difference in structure of 11-keto-17α-hydroxyprogesterone and cortisone resides in the absence of a hydroxyl group at the C-21 position in the former. However, the rate of formation of 20α-conversion product is many times slower for cortisone. It is probable, then, that hydroxylation at C-21 may contribute to ring A stability, but at the same time hinder reduction of the 20-ketone through an alteration of the spatial relationship in the steroid molecule in such a way that the points of reactivity between enzyme and substrate may be blocked. This may be further exemplified in the contrast in reactivity of progesterone and 11-desoxycorticosterone. Both compounds lack the 11-oxygen function and hence are susceptible to ring A instability. However, 11-desoxycorticosterone is at least twice as stable as progesterone to the loss of the Δ^4-3-ketone entity, the difference presumably being due to hydroxylation at C-21. On the other hand, in the absence of the accelerating 17α-hydroxyl group, and by reason of the presence of the strongly inhibiting 21-hydroxyl group, no evidence of 20α-reduction could be detected even on prolonged incubation of 11-desoxycorticosterone. Such also was the case with corticosterone. The fact that 20-keto reduction was achieved with hydrocortisone, cortisone, and Reichstein's substance S was no doubt due to the influence of the strongly activating 17α-hydroxyl group. That the spatial relationship of the various groups on the steroid molecule is the determining factor in these enzymic reactions is verified by the inability of the yeast to reduce 17β-hydroxy-17α(iso)-progesterone even on prolonged incubation.

Acknowledgments

The authors wish to thank Schering Corp., Bloomfield, N.J., for granting permission for them to obtain the microorganism and for a generous sample of 20α-dihydrocortisone. Mr. A. P. Ronald of this Station obtained the data shown in Table I.

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III. The Research Program of the International North Pacific Fisheries Commission

A. SUMMARY OF CANADIAN RESEARCH IN 1955 ON PROBLEMS RAISED BY THE PROTOCOL

Canada's part in the program of research adopted by the Commission in October, 1954, on the problems raised by the Protocol included: (1) a study of the skeletal anatomy of salmon, to seek structural differences which could be used to distinguish stocks of various origins; (2) an attempt to find parasites which can be used as indicators of the origin of salmon occurring on the high seas; (3) an attempt to catch and tag small sockeye, pink and chum salmon to indicate migrations away from rivers of origin and the fisheries to which various stocks contribute; and (4) co-operation in a study of the oceanography of the North Pacific, to provide background for understanding salmon distribution and movements. These appear in the Commission's program under items II A, II C, III 3 and IV 2 respectively. Progress in all these fields was made in 1955 and is reported in the following sections.

Canada also co-operated in other parts of the program: (a) The study of morphological and meristic characters by the United States is already showing much promise of providing the basis for distinguishing stocks of various origins. Canada assisted by supplying samples of sockeye, pink and chum salmon from Canadian waters. Samples and accompanying data were collected in northern (Skeena River), central (Rivers Inlet and Port John) and southern (Fraser River) British Columbia. (See Item II A.) (b) Blood samples of sockeye were taken at the Skeena River and at Rivers Inlet for use in the serological studies by the United States referred to in item II B of the Commission's program. (c) Assistance was given in the recovery and reporting of tags used by the United States in experiments to develop more effective methods of tagging salmon on the high seas (Item III 2 of the Commission's program).

No attempt is made here to summarize progress in the additional items of the Commission's research program undertaken by Canada at the meeting of the Commission at Tokyo, October-November 1955. These include (1) high-seas fishing as part of the co-operative attempt to sample salmon throughout their distribution, and (2) studies of scales to discover characteristics by which stocks of various origins may be distinguished. These new projects are now underway, and all the projects undertaken in 1955 are being continued and extended in 1956.

1. Skeletal Anatomy of Pacific Salmon.

The ultimate aim of this work is to find differences in bone structure by which stocks of various origins may be identified. It was, however, first necessary to study and describe the skeletal anatomy of the five species. These include *Oncorhynchus nerka* (sockeye or red), *O. gorbuscha* (pink), *O. keta* (chum), *O. kisutch* (coho or silver), and *O. tschawytscha* (spring or chinook). It was especially important to discover what changes occur as the individual salmon grows and matures; differences in structure between salmon at various stages of maturity must be distinguished from more stable hereditary differences before the latter can be used to recognize various stocks. In 1955, therefore, the objectives included (1) a thorough description of the skeletal anatomy of the five species, (2) a study of changes which occur as salmon mature, and (3) a preliminary search for skeletal characters which may be used to distinguish stocks. These phases are to be followed in 1956 by study of a few promising characters in a much greater number of samples.

Description of the skeletal anatomy of the five species. In 1955, 761 specimens were examined, taken mainly from the Fraser River area and including, insofar as possible, juveniles migrating to sea, semi-adults actively feeding at sea, maturing individuals approaching the rivers and spawning individuals. On each specimen some 40 measurements were taken, sex and stage of maturity determined, presence or absence of food in stomachs noted, and principal meristic characters counted. Colouration of specimens on the outside and in the mouth cavity was noted. Skulls and skeletons of the tail and of the paired fins were prepared and cleared in glycerine. Many hundreds of individual bones were examined and described, 32 kinds of bones being used from the skull alone. Over 500 photographs of whole fish or

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their parts were taken and about 50 X-ray pictures of heads and tails. Detailed drawings of the most characteristic bones were made.

On the basis of this material a detailed and well-illustrated description of the skeletal anatomy of the five species is in preparation.

Changes which occur as salmon mature. Individuals of any one species of Pacific salmon pass during their life span through four distinct stages—juvenile stage in fresh water and then in the sea, semi-adult feeding and growing stage, maturing stage and spawning stage. The last three can, of course, be recognized by the development of the reproductive organs. It was found that they can also be recognized by the type of teeth and the size of the bones carrying them. These are (in order of importance) the premaxillary, maxillary, supralingual, vomer, palatine and pharyngeal bones, all of which carry teeth.

In young and semi-adult specimens the teeth are sharp and sit more or less directly on the respective bones. These feeding teeth are probably replaced at least once a year. In maturing individuals, which are apparently no longer feeding actively, teeth of a new type appear. These are not yet attached to the bones but are found only in the connective tissue covering them. As the season progresses the fish become sexually mature and enter fresh water. They now have strongly developed breeding teeth sitting on large ossified bases fused to the bones. The attachment of the ossified bases greatly increases the size of the bones. The premaxillary bones are particularly affected by these changes and, therefore, especially useful for distinguishing stocks in various stages of maturity.

It is believed that a study of the teeth and jaw bones of salmon taken on the high seas can indicate the onset of maturity of various bodies of fish and help complete the picture of their movements towards the fresh waters where they spawn. Examination of extensive samples from this point of view is planned in 1956.

Differences between stocks. The work in 1955 has shown what parts of the skeleton do not change as the salmon mature and has thus laid the basis for the search in 1956 for differences between stocks. This will involve the much less detailed examination of a large number of samples.

2. Parasites of Pacific Salmon.

Studies on the parasites of Pacific salmon have been carried out in close co-operation with United States investigators who have paid particular attention to chums and some to sockeye while Canadian investigators concentrated on sockeye and pinks. All available 1955 samples have not yet been examined. The results outlined below are, therefore, subject to confirmation or adjustment. A full report on 1955 samples is planned before the 1956 Annual Meeting of the Commission.

Samples have included seaward migrants, young inshore marine salmon and adult salmon from commercial catches. Seaward migrants were obtained only from North America and young marine samples only from British Columbia. Adult salmon from commercial catches and exploratory fishing have been made available from a number of places from the Columbia River north to Alaska and west to the Sea of Okhotsk through co-operation of United States and Japanese agencies. In all over a thousand specimens have been carefully examined for parasites to date. About forty species of parasites (some of them new to science) have been found, mainly in the digestive tract, but only a few of these promise to be useful in distinguishing stocks.

Pink Salmon. The number of pinks studied to date is relatively small and from American waters only. No information of value to distinguishing pink salmon stocks has been found yet. The pink fry examined (from British Columbia and Alaska) were entirely free of parasites but young pinks in the sea were soon parasitized. Four species of parasites (three trematodes and one copepod) were found both in young pinks in the sea and in adult pinks from inshore commercial catches. It is still quite possible that valuable differences in parasitization will emerge as examination of samples from other areas proceeds.

Sockeye Salmon. More samples of sockeye have already been examined and promising results have already been found.

Three parasites (the trematode *Diplostomulum*, the cestode *Diphyllobothrium* and the nematode *Philonema*) are acquired by sockeye smolts, retained throughout their life, and return to fresh water with spawners. These parasites have a freshwater life cycle but because of their wide distribution cannot be used to distinguish Asiatic from American sock-

eye. In some cases they could be used to separate local "runs" even in the same river system.

Of the many parasites found in adult sockeye some are so generally distributed as to be useless in distinguishing stocks and others are too scarce. Table I summarizes the occurrence of certain species which offer some promise, along with some others for comparison. The unbracketed numbers represent the percentage of infected individual salmon in the sample examined; the numbers in brackets are the average numbers of parasites per infected fish (i.e. not including fish free of the parasites). Samples have now been examined from five more localities (Columbia River, Rivers Inlet and Skeena River, B. C., Petersburg, Alaska, and in the Japanese high-seas fishery at 167°50′E.) These confirm the conclusions outlined below.

TABLE I.—Incidence and intensity of parasites, by species, in adult marine sockeye, 1955.

Area	Date of Capture	Trematoda				Cestoda			Nematoda		Acanthocephala	
		Brachyphallus	*Lecithaster gibbosus*	*Hemiurus levinseni*	*Diplostomulum* sp.	*Diphyllobothrium* sp.	*Phyllobothrium cauda tum*	*Philonema oncorhynchi*	*Contracaecum*	*Anisakis* sp. larva	*Bolbosoma*	*Echinorhynchus*
Fraser R., B.C.	July 26	—	75(109)	—	—	40(3.5)	75(11)	10(42)	50(5)	95(5)	5(1)	—
Bristol Bay, Alaska	July 15	64(19)	88(12)	4(1)	4(1)	48(3)	88(15)	96(43)	72(16)	96(23)	44(1.3)	—
King Cove, Alaska Peninsula	July 7-Aug. 4	96(77)	92(122)	—	24(4)	—	92(19)	—	92(9)	100(15)	—	—
Attu, Aleutian Islands, Alaska	July 28-Aug. 9	29(2)	80(99)	21(1.6)	8(2)	—	92(13)	80(108)	62(6)	96(13)	8(3.5)	4(1)
48°43′ - 48°50′N. 170°23′ - 173°E.	May 18	27(3)	13(1)	27(1.5)	—	60(5)	87(17)	80(20)	67(2)	100(28)	80(6)	—
51°33′N., 159°04′E.	July 14	53(3)	7(1)	87(3)	—	80(3)	100(19)	73(19)	53(3)	100(26)	27(1.5)	93(7)
Okhotsk Sea	June 30-Aug. 10	64(2)	40(2.5)	40(3.5)	4(1)	64(5)	100(24)	85(40)	36(15)	100(22)	44(1.6)	76(7)

Two parasites are common in the samples examined from western areas and absent or rare in samples from eastern areas. The acanthocephalan *Echinorhynchus* was found in a high percentage of sockeye in the samples from the Okhotsk Sea and the far western Pacific (159°E.) and in smaller numbers at Attu; it was absent from all samples examined from east of Attu. The trematode *Hemiurus* was well represented in the four samples from Attu west; it was absent from all eastern areas except for a low incidence at Bristol Bay. The picture is less clear with the trematode *Lecithaster* but it has possible significance. This parasite was very abundant in all samples from Attu east but present in much smaller numbers to the west.

The above differences in the parasites of the sockeye examined to date from the eastern and western sides of the Pacific suggest that they may be used to distinguish between eastern and western stocks. More samples must be examined before definite conclusions can be drawn. Some 1955 samples remain to be examined and better coverage is planned in 1956. In the meanwhile the method appears to offer promise.

General. The results to date suggest that some species vary in abundance from north to south. The trematode *Brachyphallus*, for example, was found in all samples of sockeye from the high seas north of 48°N. but was absent from both sockeyes and pinks south of 48°N. on the high seas or south of Queen Charlotte Sound in British Columbia coastal waters.

There are some major differences between the parasites of adult sockeye and adult pinks in the same area (Fraser River, B. C.) and interpretations of data on parasites of one species are not necessarily applicable to others.

To summarize, the parasitological studies have indicated three parasites common in sockeye on one side of the Pacific but absent or rare on the other and offer some promise of a real contribution to an understanding of the distribution of Asiatic and

American salmon on the high seas. More sampling is needed and is in progress.

3. Catching and Tagging Young Salmon in the Sea.

The purpose of these studies is to discover the distribution of young sockeye, pink and chum salmon, their movements away from their rivers of origin and the fisheries to which they contribute. Methods of finding, catching, identifying and tagging them were developed with some success. Observations were also made on the growth and food of the young salmon and on the physical conditions and plankton. In 1955, young salmon were caught and some were tagged close to shore; in 1956, it is planned to follow them farther to sea and to tag larger numbers.

The investigation was carried out from June to September in two areas: (1) Chatham Sound and adjacent waters in which the seaward dispersion of young salmon from the Skeena River and various smaller streams could be explored, and (2) Johnstone, Broughton and parts of Queen Charlotte Straits where concentrations of fish from the Strait of Georgia were expected. The Fisheries Research Board vessels *Investigator No. 1* (60-foot trawler) and *Loligo* (80 feet) were used in the former, and *Alta* (a converted 40-foot troller) in the latter. Each vessel carried a small purse-seine, a beach-seine, gill-nets of various small meshes, several tow-nets of one-metre diameter and various mesh sizes, and equipment for fishing at night with lights. In the Chatham Sound area beach-seining and purse-seining were carried out from a 16-foot flat-bottomed skiff, with roller over the stern, which was powered with an 18-horse-power outboard motor set through a forward well. Preliminary experiments with a mid-water trawl were unsuccessful in catching young salmon but further attempts are planned in 1956.

Of the various types of gear used the beach-seine and purse-seine were most useful for catching small salmon. The beach-seine produced large catches (thousands of fish when abundant) and was operated successfully from all types of shore. By making circling sets it was used in waters too shallow for purse-seining and too far from shore for ordinary beach-seining. As many as 69 fish in one set were caught in this way. The purse-seine was used only over deep water and when the sea was calm. Catches pet set numbered less than 100 fish. Tow-nets, gill-nets and night-lights were used with very little success. The number of times each fishing method was used is given below.

Area	Beach seine		Purse-seine	Tow-net	Gill-net	Night-light
	Beach Set	Circle Set				
Chatham Sound	337	16	49	45	5	3
Johnstone Strait	106	2	19	2	1	3

Distribution and abundance. The results indicate the spread of young pink and chum salmon seaward. They concentrate along the shore until late July or early August when their movement away from the shore starts. In the Chatham Sound area, young sockeye were not caught as successfully as pinks and chums, perhaps because of their failure to concentrate on the beaches in the same manner. Such catches as were made suggest a similar seaward movement and disappearance from inshore areas late in the summer.

Catches of young pink salmon per set of beach-seines sometimes exceeded 5,000, and the seaward movement of concentrations along the shore of the straits and islands could be followed. Catches per set of young chum salmon did not exceed 500, of young sockeye 100 per set, but indicated similar seaward migrations.

Size. Changes during the summer in the average lengths of the young salmon caught do not indicate growth alone. This was especially true in the Johnstone Strait area through which salmon of the tributaries to the Strait of Georgia pass. Here fluctuations in length suggested the passage of stocks from different streams or reaching the sea at different times. In the Chatham Sound area pink salmon caught in June averaged about 65 mm. in length, those caught in September about 125 mm. Chum salmon showed somewhat similar increases in length. In general the sockeye caught in June were longer (about 78 mm.); the few caught in September averaged about 100 mm. The actual growth is probably larger than these figures indicate for all three species, the older larger fish probably leaving the inshore

areas earlier. In all three species females were slightly larger than males.

Experimental tagging of small salmon. In experimental tagging of 3- to 4-inch salmon, several types of tags were tested. These included: (a) plastic sleeves, 0.039 inch in diameter and 0.25 inch in length, attached with No. 26 gauge silver wire or 15-pound test monofilament nylon, (b) toggle tag which consisted of two small plastic balls threaded on nylon which was wound into twisted wire, (c) hook tag with streamer, and (d) 5- to 6-inch lengths of plastic "spaghetti" tubing of 0.039-inch diameter, the free ends knotted to form a small loop. After holding the tagged fish in aquaria for eleven months, some information on the relative merits of each type is available.

Toggle and hook tags, while easy to apply, caused irritation at the point of attachment shortly after tagging. The maximum periods for which these tags remained attached were 96 days and 154 days respectively. Wire tags showed considerable cutting of the flesh. Eleven of the 13 fish with this tag died within 290 days, 8 of the 11 within 70 days. Fish with monofilament nylon and "spaghetti" tags show the best survival to date (approximately 40% or roughly one-half the survival of the control fish) and some of the losses are attributed to accident. The "spaghetti" tag has the additional advantage of easy application, and therefore appears most promising.

To indicate the survival of small fish tagged with different types of tag, 722 sockeye smolts were tagged with type (a) tag with wire and 597 smolts with type (d) tag in early June at the counting fence at Lakelse River on the Skeena watershed. Some information may be available in 1956 when some of the fish return as "jacks."

Tagging in the field. A total of 4,222 small salmon were tagged mainly with "spaghetti" tags of polyethlyene tubing and some with plastic sleeves attached with wire.

Little immediate mortality was observed after tagging. Three chum salmon, released in Alert Bay, were recovered in the same area one to three days later. A coho tagged in Hardy Bay was recovered eight days later and six miles away in a commercial purse-seine.

Food. Over 90% of the 1,577 stomachs examined contained food. Important items in the food were copepods, larvacea, amphipods and euphausiids and, for coho, fish. Differences in feeding habits were indicated. In general pink, chum and sockeye were eating invertebrates, mainly small crustacea, while even at this early age coho were fish eaters. When pink and chum salmon occurred together the chum appeared to take a greater variety of food organisms.

Relation of oceanographic conditions. Two water masses were recognizable in Chatham Sound on the basis of volumes and composition of zooplankton and of temperatures and salinities. One is a shoreward movement of cold oceanic water rich in copepods. The other is plankton-poor, warmer and more brackish as a result of fresh water from the Skeena and Nass Rivers. The young salmon were found most frequently in the latter during the period of this investigation.

4. Oceanography.

Knowledge of the oceanography of the North Pacific is essential to an understanding of the distribution and movements of salmon. In July to September, 1955, oceanographic agencies of Japan, United States and Canada co-operated in a survey of the Pacific north of latitude 20°N. known as Project *Norpac*. All agencies exchanged data records in February, 1956 and further analysis and publication is proceeding. This great survey, although initiated under other auspices, is of great value to the Commission's research program on the problems raised by the Protocol and this value was an important consideration in Canada's active participation.

Species	Chatham Sound "spaghetti" tag	Johnstone Strait "spaghetti" wire		Total
Pink	614	114	61	789
Chum	129	497	98	724
Sockeye	—	1,945	154	2,099
Coho	48	284	243	575
Spring	—	5	30	35
Total	791	2,845	586	4,222

Canada's part of the *Norpac* survey was carried out by the frigate H.M.C.S. *Ste. Therese* (Fig. 1) which cruised 7,160 miles between July 26 and September 1 in the area between latitude 47°N. and

Figure 2 shows the surface temperature distribution in August, 1955, and indicates the circulation. In a zone between latitudes 40°N. and 50°N. there is a relatively rapid change in surface temper-

Fig. 1. H.M.C.S. "*Ste. Therese*," naval frigate provided by the Royal Canadian Navy and equipped to conduct oceanographic surveys in North Pacific waters for Project Norpac, August, 1955, as part of the INPFC research program for 1955.

the Aleutian Islands and Alaska, and between the North American coast and longitude 177°W. At 85 oceanographic stations temperatures and water samples were taken to a depth of 600 fathoms. Data were obtained on temperature, salinity, dissolved oxygen, and inorganic phosphate. Vertical plankton hauls were taken at 50-fathom intervals from the surface to 200 fathoms. Bathythermograph lowerings to 150 fathoms were made at each station and between stations, obtaining a continuous record of the changes of temperature with depth.

The data obtained are being analyzed in close co-operation with United States agencies, especially the Oceanographic Labbratories of the University of Washington, which also made observations in the Northeast Pacific and Gulf of Alaska. The areas covered are particularly important to distribution of salmon in the eastern North Pacific and it is planned to prepare a joint report for presentation to the Commission before its next Annual Meeting. Analysis of the data is still proceeding and space does not permit more than a brief mention of some of the results which are emerging. The data presented in the figures came from both United States and Canadian operations.

ature from south to north. South of this zone in mid-Pacific and north of it in the Gulf of Alaska, surface temperatures vary little over large areas. The zone of rapid change in temperature is also the zone where the eastward current is best developed. North of this zone the currents are in general slower and less well defined. The Gulf of Alaska is shown as an area with a slow contra-clockwise circulation and with relatively little variation in surface temperatures from place to place. It is, as it were, a great eddy with characteristics which distinguish it from the waters to the south. Recent United States exploratory fishing indicates a southern boundary for salmon and a northern boundary for tuna lying in the zone of strong eastward flow. This relates salmon distribution to the water mass north of this zone and directs attention to its nature and its extent.

Figure 3 shows the salinities along longitude 147°W. It shows a shallow surface layer of low-salinity water lying from the temperature transition zone northward. This water mass is distinct from the more saline oceanic surface water to the south. Earlier observations in the areas off the British Columbia coast indicate that this low-salinity surface layer is present throughout the year whereas temperatures, of course, vary seasonally. The intensified

exploratory fishing and oceanographic work planned for 1956 will throw light on the relationship of salmon distribution to this water mass and, it is hoped, indicate more definitely whether it is indeed "salmon water."

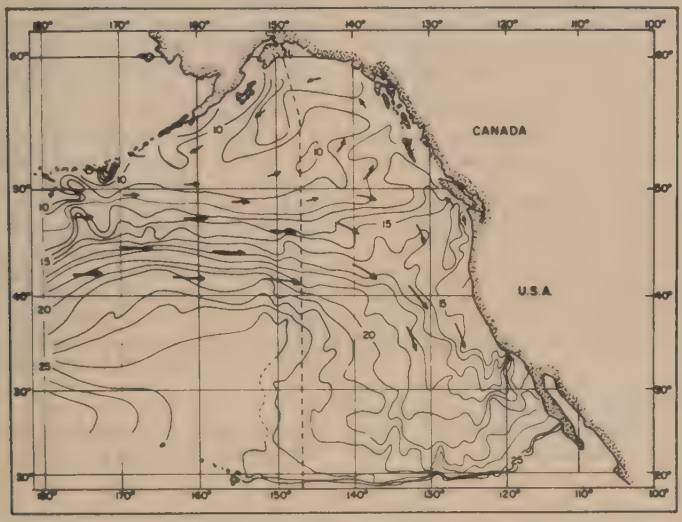

Fig. 2. Surface temperatures (in degrees Centigrade) and water movements in the Northeast Pacific, August, 1955, from results of the Norpac survey.

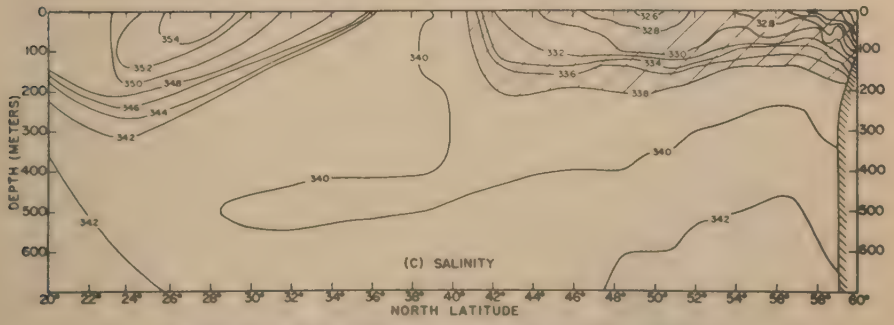

Fig. 3. Salinities in the North Pacific in a section along Longitude 147° W.

B. A SUMMARY OF CANADIAN RESEARCH IN 1956 ON PROBLEMS RAISED BY THE PROTOCOL

In 1956 work was continued and extended on projects underway in 1955, including a study of the skeletal anatomy of salmon; an attempt to find parasites which will indicate the origin of salmon occurring on the high seas; tagging of small sockeye, pink and chum salmon to indicate migrations away from rivers of origin and the fisheries to which various stocks contribute; and co-operation in a study of the oceanographic background for salmon distribution. These appear as Items II A, II C, III 3, and IV 2 in the program of research on the problems raised by the Protocol adopted by the Commission in October, 1954. Work was initiated in 1956 on two additional items undertaken by Canada at the meeting of the Commission at Tokyo, October-November, 1955, namely, participation in high-seas fishing to sample salmon and in studies of scales to discover characteristics by which stocks of various origins may be distinguished.

Canada also co-operated in other parts of the program, (a) by supplying samples of sockeye, pink and chum salmon from inshore Canadian waters for use in other projects, including study of morphological and meristic characters and serological studies, and (b) by assistance in the recovery and reporting of tags.

Progress was made in 1956 in all aspects of the work and preparations have been made for continuation of all the above projects in 1957.

1. Sampling of Salmon on the High Seas.

During the summer of 1956, two chartered vessels, the *Key West II* and the *Challenger*, conducted salmon-fishing operations on the high seas of the northeast Pacific. The positions of fishing stations are shown in Figure 1. Catches and other data are summarized in Table 1.

Fishing was performed with gill-nets of four mesh sizes distributed in the following proportions: 5¼" - 3 shackles; 4½" - 1 shackle; 3¼" - 1 shackle; 2½" - 1 shackle. Each shackle was 50 fathoms long.

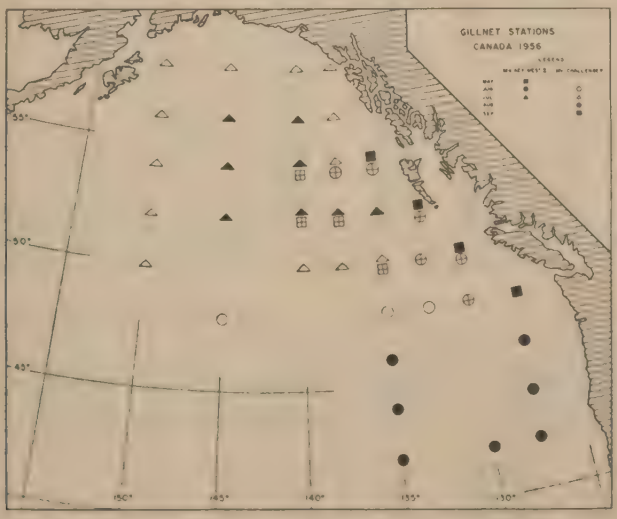

Fig. 1. Gillnet stations occupied by Canadian research vessels, 1956.

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FRB No. 655

TABLE 1. Salmon catches in the northeast Pacific by Canadian research vessels in 1956.

Date	Position Lat. N.	Long. W.	No. of shackles	Fishing time (hr.)	Surface temp. (°C.)	Species Sockeye	Pink	Chum	Coho	Chinook	Total
					Challenger						
June 3	48°	132° 30′	6	8:15	10.8	—	13	—	3	—	16
4	48	135	6	10:00	9.6	1	—	1	2	—	4
7	48	145	6	10:30	7.6	3	1	1	3	2	10
July 8	54	137° 30′	6	10:00	11.5	1	—	3	5	—	9
9	56	137	18	9:00	12.0	9	4	34	18	1	66
10	58	137	18	6:30	11.0	21	20	31	10	—	82
11	58	140	18	7:15	11.0	16	5	12	10	—	43
12	58	145	18	6:45	12.0	18	24	1	21	—	64
13*	58	150	18	9:45	9.0	1	—	19	1	—	21
17	56	150	18	9:30	12.1	31	30	42	8	—	111
18	54	150	18	9:30	12.1	10	19	15	7	1	52
19	52	150	18	6:15	9.3	5	7	7	2	—	21
20	50	150	18	11:45	10.5	17	1	8	—	—	26
23*	50	140	18	11:30	13.0	—	—	—	—	—	0
24	50	140	18	12:15	12.5	—	—	—	—	—	0
25	50	137° 30′	18	11:45	13.0	—	—	—	—	—	0
26	50	135	18	12:00	13.5	—	—	—	—	—	0
Aug. 12	50	130	24	7:00	15.0	—	—	—	—	—	0
15	52	132	24	8:15	14.0	—	3	1	—	—	4
16*	54	135	24	5:45	12.5	—	1	—	—	—	1
22	54	135	24	12:00	14.0	—	56	55	17	—	128
23	54	137° 30′	24	9:30	14.0	—	1	—	—	—	1
25	50	132° 30′	24	8:00	16.0	—	—	—	—	—	0
26	48	130	24	6:00	18.0	—	—	—	—	—	0
Sept. 6*	54	140	24	7:45	12.9	—	1	—	—	—	1
7	54	140	24	10:45	12.9	—	—	6	—	—	6
8	52	140	24	8:30	13.0	—	—	—	—	—	0
9	52	137° 30′	24	11:45	14.0	—	—	—	—	—	0
10	50	135	24	8:15	14.3	—	—	—	—	—	0
					Key West II						
May 23	54	135	12	10:30	8.0	5	—	—	—	—	5
28	52	132	18	7:50	9.4	10	4	10	1	—	25
29	50	130	12	8:00	11.1	—	4	—	1	—	5
31	48	127° 30′	18	6:30	12.5	1	1	—	—	—	2
June 18	46	135	12	8:20	11.0	—	2	—	1	—	3
19	44	135	12	9:00	12.1	—	—	—	—	—	0
20	42	135	6	9:50	13.0	—	—	—	—	—	0
21	42	130	6	4:45	13.0	—	—	—	—	—	0
22	42	127° 35′	12	8:45	14.7	—	—	—	—	—	0
26	44	127	12	8:45	14.8	—	—	—	—	—	0
27	46	127	12	9:20	14.2	—	—	—	—	—	0
July 12	56	140	18	7:50	10.2	5	6	—	7	—	18
13	56	145	18	9:10	11.0	7	18	8	11	—	44
14	54	145	18	8:50	11.4	6	6	36	5	—	53
15	52	145	12	9:15	11.0	7	3	11	3	1	25
17	52	140	12	9:30	12.0	11	3	9	—	—	23
18	54	140	18	9:25	12.0	2	10	21	—	—	33
19	52	137° 50′	12	10:10	12.2	—	2	1	—	—	3
20	52	135	18	10:40	13.3	13	5	17	3	—	38
Total						200	250	349	139	5	943
Per cent.						21.2	26.5	37.0	14.7	0.5	99.9

* Day-time set.

TABLE 2. Salmon catch per unit of gear.

Mesh Size	Number shackles fished	Sockeye		Pink		Chum		Coho		Spring		Total Salmon Catch	
		No. fish	Catch per shackle	No. fish	Catch per shackle	No. fish	Catch per shackle	No. fish	Catch per shackle	No. fish	Catch per shackle		
2½″	134	35	.26	0	0	23	.17	12	.89	0	0	70	.52
3¼″	134	15	.11	33	.24	81	.60	7	.52	0	0	136	1.01
4½″	134	30	.22	94	.70	73	.54	28	.20	2	.01	227	1.69
5¼″	402	120	.30	123	.30	172	.42	92	.22	3	.007	510	1.26

TABLE 3. Length and weight of sockeye salmon caught in the northeast Pacific by Canadian research vessels, 1956, according to age and sex.

Age	Male				Female			
	Length (cm.)		Weight (kg.)		Length (cm.)		Weight (kg.)	
	Number measured	mean	Number weighed	mean	Number measured	mean	Number weighed	mean
2_1	—	—	—	—	2	34.5	2	2.1
3_1	2	41.5	2	2.9	4	51.0	4	1.9
3_2	21	39.7	18	1.0	14	39.5	11	1.2
4_1	1	60.0	1	3.8	1	61.0	1	3.8
4_2	40	53.1	39	2.4	40	56.6	39	2.5
4_3	3	44.7	3	1.4	—	—	—	—
5_2	10	61.7	8	2.9	16	59.4	15	3.2
5_3	5	59.0	5	3.1	10	55.1	10	2.5
6_3	3	61.0	3	3.4	4	60.3	4	2.8

TABLE 4. Age and sex composition of sockeye salmon caught in the northeast Pacific by Canadian research vessels, 1956.

Age	Male		Female		Sex unknown		Sexes Combined	
	No.	%	No.	%	No.	%	No.	%
2_1	—	—	2	1.0	—	—	2	1.0
3_1	2	1.0	4	2.0	—	—	6	3.0
3_2	21	10.5	14	7.0	3	1.5	38	19.0
3_3	—	—	—	—	1	0.5	1	0.5
4_1	1	0.5	1	0.5	—	—	2	1.0
4_2	40	20.0	40	20.0	3	1.5	83	41.5
4_3	3	1.5	—	—	—	—	3	1.5
5_2	10	5.0	16	8.0	1	0.5	27	13.5
5_3	5	2.5	10	5.0	—	—	15	7.5
6_3	3	1.5	4	2.0	—	—	7	3.5
?	4	2.0	4	2.0	8	4.0	16	8.0
All ages combined	89	44.5	95	47.5	16	8.0	200	100.0

TABLE 5. Length and weight of chum salmon caught in the northeast Pacific by Canadian research vessels, 1956, according to age and sex.

Age	Male				Female			
	Length (cm.)		Weight (kg.)		Length (cm.)		Weight (kg.)	
	Number measured	mean	Number weighed	mean	Number measured	mean	Number weighed	mean
2	21	38.3	19	0.8	30	37.6	26	0.7
3	94	53.4	91	2.2	71	52.8	71	2.1
4	35	62.5	36	3.0	67	57.9	64	2.9
5	1	61.0	1	4.1	2	59.0	1	3.8

TABLE 6. Age and sex composition of chum salmon caught in the northeast Pacific by Canadian research vessels, 1956.

Age	Male		Female		Sex unknown		Sexes Combined	
	No.	%	No.	%	No.	%	No.	%
2	21	6.0	30	8.6	8	2.3	59	16.9
3	93	26.7	71	20.3	7	2.0	171	49.0
4	36	10.3	66	18.9	3	0.9	105	30.1
5	1	0.3	1	0.3	—	—	2	0.6
?	1	0.3	7	2.0	4	1.1	12	3.4
All ages combined	152	43.6	175	50.1	22	6.3	349	100.0

TABLE 7. Length and weight of pink salmon caught in the northeast Pacific by Canadian research vessels, 1956, according to sex.

Sex	Length (cm.)		Weight (kg.)	
	Number measured	mean	Number weighed	mean
Male	124	49.7	120	1.81
Female	101	47.9	97	1.67

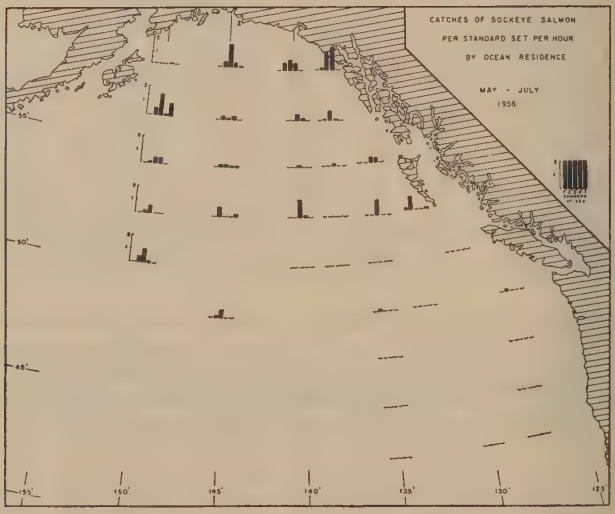

Fig. 2. Sockeye salmon catches during May - July, 1956.

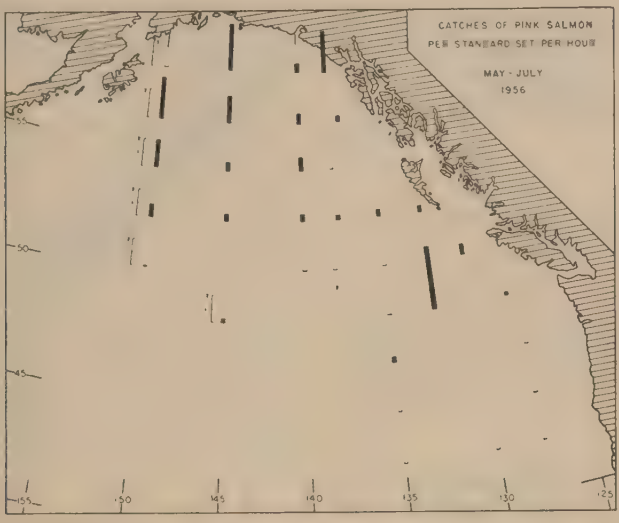

Fig. 3. Pink salmon catches during May - July, 1956.

Fig. 4. Chum salmon catches during May - July, 1956.

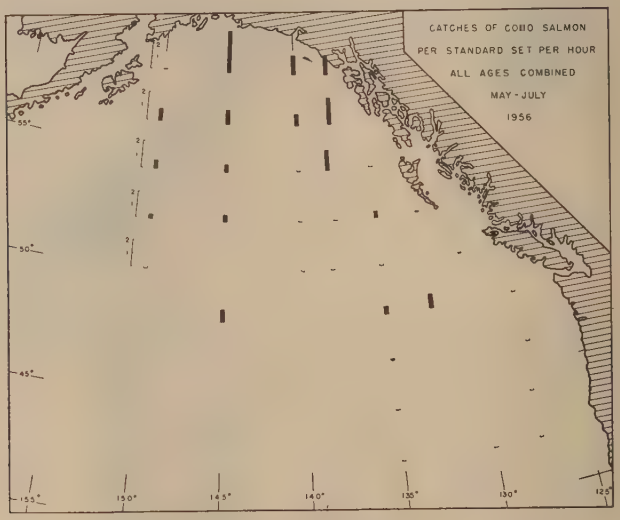

Fig. 5. Coho salmon catches during May - July, 1956.

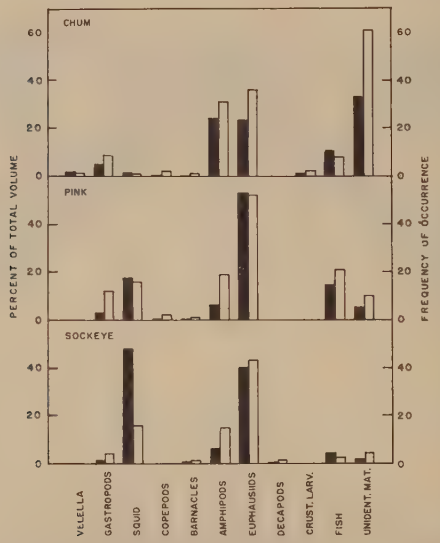

Fig. 6. Stomach contents of salmon. Solid bars: per cent. volume. Plain bars: frequency of occurrence.

The total number of shackles used for each set is given in Table 1. Nets were set out over a stern roller and were hauled in amidships by means of a power block.

Refrigeration equipment, consisting of a quick-freezing brine tank and cold storage boxes of 5,000-pound capacity, was installed on each vessel.

Salmon catches. The distribution of catches according to size of mesh is shown in Table 2. The 4½" mesh showed the greatest overall efficiency, but this was not true for each separate species.

Recorded data on the position of enmeshed fish in the nets showed that in sets made at night a majority of the salmon were caught in the upper half of the nets (total depth of nets was about 20 feet). In rough weather this preponderance was reduced and in the few sets made in the daytime more fish were caught in the lower portion of the nets.

Tables 3 to 7 give statistics of the sockeyes, pinks and chums constituting the catches.

toward fresh water. Furthermore, the southern limit of feeding fish appeared to shift nothward with the advance of the summer season—at least as regards the fish available to surface nets. Thus, while salmon were caught at Latitude 46° in June, they were only taken north of Latitude 50° in July and August (see Table 1).

Food. Stomachs have been examined of sockeye (190), pink (96) and chum (125). Of these, 24% of the sockeye stomachs, 56% of the pink stomachs and 55% of the chum stomachs contained food. The main categories of organisms in these stomachs are shown in Figure 6. While the three species of salmon show quantitative differences in the proportions of various organisms consumed, it is even more evident that there is much similarity in the diet.

Environmental conditions. In conjunction with fishing operations the following observations and samples were taken:

	Bathythermograph casts	Salinity samples	Total phosphate samples	Plankton samples	
				Vertical	Horizontal
Number of observations	202	582	202	195	44

Distribution of species. The wide general distribution of sockeye, pink, chum and coho salmon is shown in Figures 2 to 5. Certain changes in the distribution picture took place during the course of the summer. In July and August, mature fish became scarce in high-seas catches because of their migration

Factors influencing distribution. Table 8 shows that salmon were caught at surface temperatures ranging from 7°C. to 14°C. Average catches were reduced at temperatures above 12° and no salmon were caught when the temperature exceeded 14°. The findings were similar for all species.

TABLE 8. Catch of salmon in northeast Pacific, 1956, in relation to surface water temperatures.

		Sockeye		Pink		Chum		Coho		Spring	
Temp. °C.	No. of total sets	No. of sets with fish 1	Av. No. fish/ set/ hr. 2	No. of sets with fish 1	Av. No. fish/ set/ hr. 2	No. of sets with fish 1	Av. No. fish/ set/ hr. 2	No. of sets with fish 1	Av. No. fish/ set/ hr. 2	No. of sets with fish 1	Av. No. fish/ set/ hr. 2
7	1	1	0.86	1	0.29	1	0.29	1	0.86	1	0.57
8	1	1	0.73	—	—	—	—	—	—	—	—
9	4	4	0.62	2	0.41	4	1.17	4	0.29	—	0.04
10	3	2	0.70	3	1.86	1	0.23	2	0.66	—	—
11	8	6	1.04	7	1.00	6	1.77	8	0.88	1	0.02
12	13	7	0.78	10	0.87	8	1.16	4	0.52	2	0.01
13	7	1	0.17	1	0.07	1	0.23	1	0.04	—	—
14	8	—	—	3	0.48	2	0.43	1	0.13	—	—
15	1	—	—	—	—	—	—	—	—	—	—
16	1	—	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—
18	1	—	—	—	—	—	—	—	—	—	—

Observed surface salinity values varied from 31.89‰ to 33.15‰. No correlation was found between catches and salinities within this range.

Miscellaneous catches. In addition to Pacific salmon, various other species of fishes, also squid, appeared from time to time in the nets (Table 9). It was of interest to find that the steelhead *(Salmo gairdneri)* was as widely distributed as the Pacific salmons in the region covered by Canadian fishing operations.

2. Distribution and Tagging of Juvenile Salmon.

In continuation of studies begun in 1955, the distribution and growth of young salmon during the early part of their marine life were investigated in the Chatham Sound - Dixon Entrance area of the British Columbia coast.

From early July to early September, fishing operations were carried out by the chartered drum-seiner *Cape Blanco*. In July, sampling of the young salmon populations was effected mainly by beach seining, but with the progressive movement of the fish away from the shorelines the purse-seine was used almost exclusively.

Figure 7 shows the areas and localities in which operations were conducted. The species composition of the catches was determined from samples which were preserved from each successful set. The remaining fish of the catches were tagged or, if too small

TABLE 9. Miscellaneous catches.

Species	Number of occurrences	Total number caught
Steelhead	16	34
Pomfret	16	504
Scad	10	112
Squid	10	54
Albacore	1	1
Rockfish	1	17
Dogfish	2	18
Blue Shark	14	55
Mackerel Shark	6	11

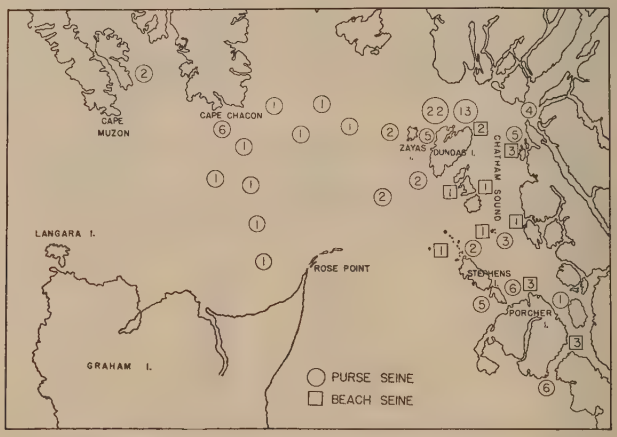

Fig. 7. Localities fished for juvenile salmon. Figures indicate number of sets made.

or otherwise unsuitable for this purpose, were released. Among approximately 10,000 juveniles which were either tagged or preserved, the species were represented in the following proportions: sockeye, 1.5%; pink, 87%; chum, 10%; coho, 1.5%.

The preserved samples were used to record changes in the length of pink and chum salmon captured during a two-month period beginning early in July (Figures 8, 9). Numbers of preserved sockeye and coho were insufficient to show progressive changes in size at short-time intervals. A total length range (fork length) from 7.5 cm. to 22.5 cm. was recorded for juvenile sockeye and from 8.2 cm. to 30.7 cm. for juvenile coho.

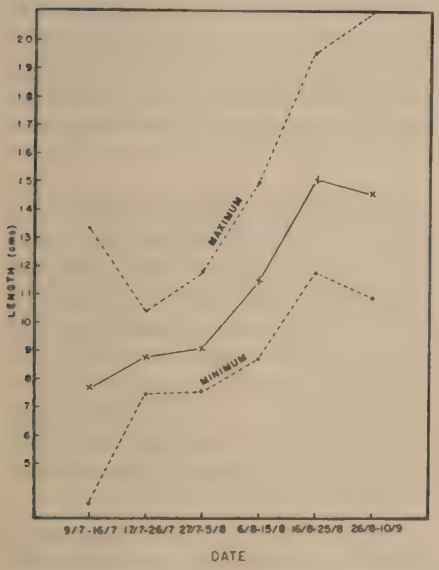

Fig. 8. Changes in size (fork length) of pink salmon, July 9 to September 10, 1956.

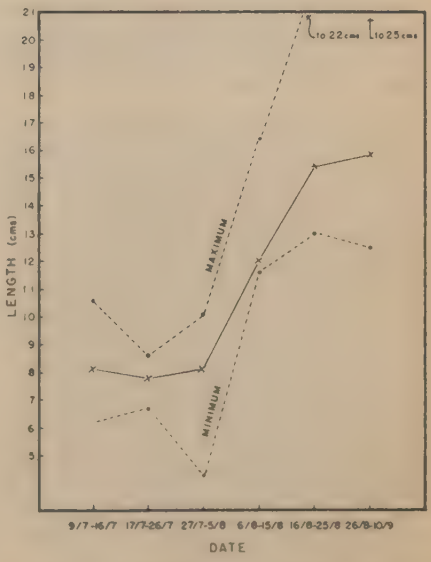

Fig. 9. Changes in size (fork length) of chum salmon, July 9 to September 10, 1956.

Tagging. A total of 7,717 juvenile salmon were tagged with "spaghetti" tags, the distribution being: sockeye, 9; pink, 6,629; chum, 900; coho, 179.

Five of these tags which had been applied at Dundas Island in late August and early September were recovered during the latter month, all being taken from the stomachs of large cohoes. In two instances the recoveries were made in Alaskan waters.

3. Skeletal Anatomy of Pacific Salmon.

This study was planned in two phases: (1) a description of the osteology of the principal species of Pacific salmon and of the changes which take place as maturity approaches, and (2) a search for differences in bone structure between stocks of the same species but of different river origin. The first phase is a prerequisite for the second as it is necessary to distinguish between the normal differences in bone structure associated with various stages of development and the differences which can be used to distinguish stocks.

In 1956 the first phase approached completion. The results consist of detailed descriptions which are difficult to summarize but which it is intended to publish as soon as possible.

Definite differences were found in the skeletal anatomy of the five principal species which can readily be distinguished by their osteology. Individuals of any one species pass through four stages differing in skeletal anatomy: (1) juvenile, (2) semi-adult and actively growing, (3) maturing, and (4) sexually mature. These stages can be identified by changes in the teeth and associated bones—premaxillary, maxillary, supralingual, vomer, palatine and pharyngeal. In young and semi-adult specimens the teeth are sharp and set more or less directly on the bones, and these **feeding teeth** may be replaced during active growth. In maturing individuals a new type of tooth appears, not attached to the bone but held firmly in the connective tissue. As the fish become mature these **breeding teeth** are set on large ossified bases which fuse to increase the size of the bones. The premaxillary bones are especially useful for indicating the onset of maturity.

A start was made in 1956 in the second phase of the study by examining certain head bones and caudal fin structures of sockeye, pink and chum salmon from a number of sample areas. As this report was written, adequate samples, especially from the western areas, were not yet available and it is not yet possible to present results on the recognition of stocks of various origins.

4. Use of Parasites to Distinguish Stocks.

The annual report for 1955 summarized results of the examination of part of the sockeye and pink salmon samples collected in 1955 and indicated that several parasites might be useful in distinguishing between Asiatic and North American stocks of salmon on the high seas. The present report summarizes results of examination of all 1955 samples and of a few collected in 1956. Many more sockeye samples and practically all the pink samples taken in 1956 have yet to be examined.

The total number of samples collected in 1956 is about three times the number examined in 1955. They comprise adult sockeyes and pinks from all major North American coastal areas and from high-seas stations spaced when possible at 5° intervals of longitude from North America to the Sea of Okhotsk and in the Bering Sea. Pinks from Hokkaido were also made available. Sockeye smolts from North America were again examined but pink fry were not studied because the 1955 results indicated that they are free or about free of parasites. The sample size of each species at each locality was usually 25 fish. United States and Japanese agencies have again co-operated in supplying the samples.

In 1955, 1,509 salmon were examined (966 sockeyes and 543 pinks). The sockeyes included 476 smolts, 92 marine juveniles and 398 adults. The smolts were taken from 13 localities from Alaska to the Columbia River. All marine juveniles were taken at one locality in British Columbia. The adult sockeyes were taken on the high seas (mainly in the western Pacific but also in eastern areas) and from 11 North American coastal regions, extending from Attu Island to Bristol Bay, Alaska and south to the Columbia River.

The pink salmon consisted of 100 fry, 74 juveniles and 349 adults. The fry were taken from two localities (one each in British Columbia and Alaska); the marine juveniles from two localities in British

Columbia. The adults were, with few exceptions, from essentially the same areas as the adult sockeye.

More than 50 species of parasites were encountered, some of which could not be identified because of the poor condition of the available specimens, but these were species of rare occurrence. Most of the parasites occurred in the alimentary tract, but some were taken from the muscle, body cavity, eyes, gills, skin and swim bladder. Forty-five species were identified, at least generically, of which two were monogenetic trematodes, 14 were digenetic trematodes, 9 were cestodes, 6 were acanthocephalans, 9 were nematodes, 4 were copepods, and 1 was protozoan. Only a few of the parasites offer promise in distinguishing between stocks of salmon of North American and Asiatic origin. A few others may be useful in distinguishing local stocks or identifying the origin of individual fish.

Sockeye Salmon. Forty-two identified species were found parasitic in sockeyes, of which 16 were acquired in fresh water and 24 in the sea. The origin of 2 species found in salmon taken in the sea is unknown. Of the known freshwater species, 4 were found to persist throughout the life of the fish. A fifth species found in adults seems to be of freshwater origin but it was not found in any of the smolts examined.

Parasites of freshwater origin retained throughout the life of the salmon would be ideal for distinguishing stocks of various origins if their distribution were restricted. Three of the four parasites with a life cycle of this type (*Diplostomulum*, a larval trematode; *Diphyllobothrium*, a larval cestode, and *Philonema*, a nematode) are more or less widely distributed and hence cannot be used to distinguish eastern from western stocks generally. However, since their distribution and abundance is not uniform throughout all salmon populations, they may be used to separate local populations. The fourth species, *Triaenophorus crassus*, is a larval cestode found in the musculature and has a very limited distribution. The adult stage of this parasite is found only in the pike (*Esox lucius*) and the presence of *Triaenophorus* in sockeye is dependent on the coexistence of young sockeye and pike in the same watershed. As far as is known, particularly in North America, pike are absent from most sockeye spawning areas. *Triaenophorus* was found in smolts and adults of only two localities, both in the Bristol Bay area. In Lake Aleknagik (or Wood River) 36% and 52% of the 1955 and 1956 smolt samples, respectively, carried *Triaenophorus*. Thirty-six per cent. of the adult sample from this locality in 1956 were infected with *Triaenophorus*. Smolts and adults from the Naknek River showed an incidence of 16% and 4%, respectively, with *Triaenophorus*. Two sockeye, with *Triaenophorus*, were taken on the high seas in 1956, and our present knowledge suggests that these were of Bristol Bay origin. One of the two was taken at 56°N., 150°W. (Gulf of Alaska) and the other was taken at 51°N., 175°E.

The 1955 samples indicated that of the 24 marine-acquired parasites, 4 showed differences in occurrence between eastern and western samples, and might be useful in distinguishing between stocks of eastern and western origins. However, because all of the western samples were from the high seas and most of the eastern samples were from coastal areas, the possibility was entertained that the differences observed in the distribution of one of the parasites was actually due to onshore-offshore differences rather than true east-west differences. The information gathered from the 1956 samples studied so far seems to support this view. The four parasites will be discussed individually.

(1) *Hemiurus levinseni* (Fig. 10). This stomach trematode was present in 21% to 56% of the 5 samples taken in 1955 from Attu Island to the Sea of Okhotsk, with an average of 2 to 4 per infected fish. Of the 12 areas sampled in 1955 from the Alaska Peninsula to the Columbia River, the parasite was found but once in each of 3 localities. Of the 1956 samples, the parasite was encountered in 4% to 8% of the fish from 3 of 17 localities east of 175°E. longitude. These comprised nine coastal areas (one with 2 infected sockeye) and 8 high-seas stations, including one in the Bering Sea. The parasite thus has a much greater abundance in sockeye from western waters. The distribution of *H. levinseni* in the 1955 samples is shown graphically in Fig. 10. In this and other figures the incidence of occurrence (i.e., the percentage of the sample infected, irrespective of the number of parasites per infected fish) is plotted by area.

(2) *Tubulovesicula lindbergi* (Fig. 10). In 1955 this stomach trematode was present in small numbers

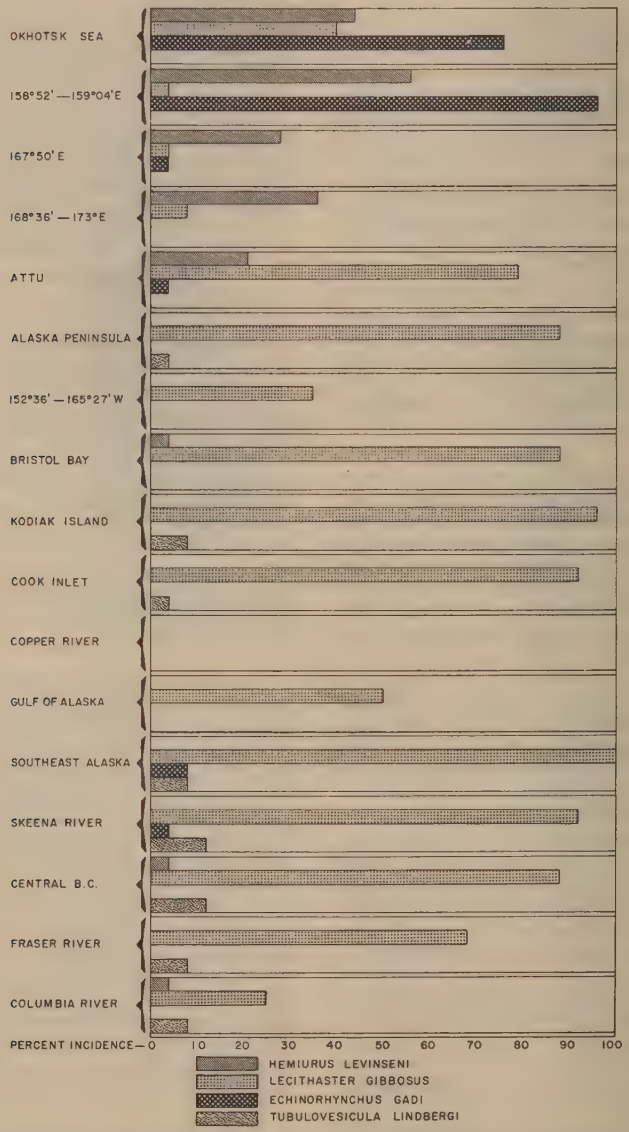

Fig. 10. Per cent. incidence of certain parasites of sockeye salmon in different areas (1956 samples).

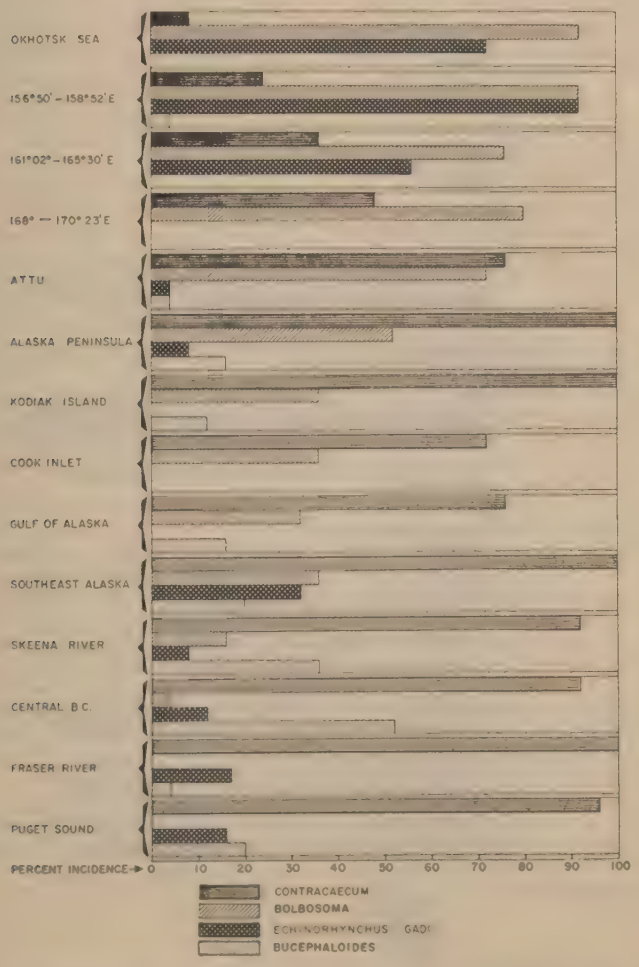

Fig. 11. Per cent. incidence of *Contracaecum*, *Bolbosoma*, *Echinorhynchus* and *Bucephaloides* in pink salmon in different areas (1955 samples).

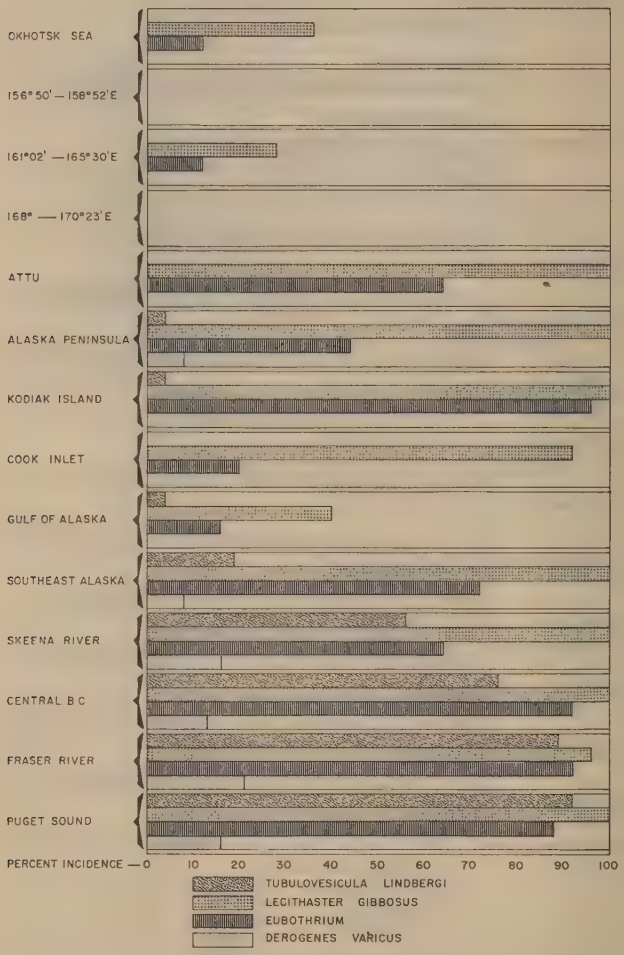

Fig. 12. Per cent. incidence of *Tubulovesicula*, *Lecithaster*, *Eubothrium* and *Derogenes* in pink salmon in different areas (1955 samples).

in 8 of 12 samples from eastern waters and entirely absent from 5 samples from Attu to the Sea of Okhotsk. In 1956 it was again found, sometimes in greater numbers than in 1955, in 10 of 17 localities from 175°E. longitude to the North American coast. The literature on fish parasites in Pacific Asiatic waters does not indicate that this parasite has been found in salmon. The presence of this parasite seems to indicate sockeye of eastern origin.

(3) *Echinorhynchus gadi* (Fig. 10). In 1955 this acanthocephalan was very common in the Sea of Okhotsk and the most westerly (159°E.) of the Pacific high-seas samples. Between 161° - 165°E. and at Attu it was observed but once. East of Attu the parasite was absent from 10 of 12 areas and was rarely encountered in the adjacent areas of southeastern Alaska and the Skeena River, British Columbia. The parasite was absent from all sockeye studied in 1956 from 17 localities east of 175°E. longitude. The value of this parasite in distinguishing between eastern and western sockeye stocks is not clear, but it was extremely characteristic of far-western samples.

(4) *Lecithaster gibbosus*. This intestinal trematode (Fig. 10) is mentioned here since in the 1955 report it was selected as having possible significance. In the coastal samples from North America in 1956 the parasite was again found to be very common, but in the high-seas samples from 175°E. to 140°W. longitude the incidence and intensity of occurrence approaches that of the western high-seas samples in 1955. It is suggested that differences in occurrence are onshore-offshore rather than east-west. Examination of the remainder of the 1956 samples should clarify the picture.

Pink salmon. Twenty-seven species of parasites were recovered from pinks, of which 25 were of marine origin, 1 is possibly of freshwater origin, and 1 unknown. Eight of the marine-acquired parasites show, to a greater or lesser degree, substantial differences in incidence between eastern and western stocks and may prove useful in distinguishing these stocks.

(1) *Bolbosoma* sp. (Fig. 11). This post-larval acanthocephalan is found in the intestines. In the 1955 samples it was present in 72% to 92% of the 5 samples from Attu to the Sea of Okhotsk, with an average of 5 to 21 per infected fish. From the Alaska Peninsula eastward and southward the incidence decreased progressivly from 52% to complete absence at the Fraser River and Puget Sound, Washington. The average number of parasites per infected fish in the eastern areas was from 1 to 3. Abundance of *Bolbosoma* thus seems to be characteristic of western stocks.

(2) *Contracaecum* (Fig. 11). The distribution of infection with *Contracaecum* was the reverse of that of *Bolbosoma*. In all areas from Attu to the east the incidence of *Contracaecum* was between 72% and 100%. West of Attu the incidence decreased progressively from 48% to 8% in the Sea of Okhotsk. Abundance of *Contracaecum* was associated with eastern stocks.

(3) *Echinorhynchus gadi* (Fig. 11). A high abundance of this parasite in the three most westerly areas was evident in 1955. It occurred in 56% to 92% of the samples, with an average intensity of 3 to 5 per fish in the infected groups. From Attu east, the parasite was present in 3 of 10 areas but the incidence was less than 20%, except in southeast Alaska where it was 32%. The average intensity in the eastern areas was 1 to 3 per fish in the infected groups. The absence of *Echinorhynchus* from the sample taken between 168°E. and 170°E. is not promising for the use of this parasite as a means of distinguishing eastern and western stocks of pinks. However, the high incidence in the three most westerly areas seems to be of significance.

(4) *Bucephaloides* sp. (Fig. 11). This intestinal trematode appears essentially to be a parasite of eastern stocks of pinks. It was found in only 2 fish of the 5 samples from Attu to the Sea of Okhotsk. Of the 9 samples from the Alaska Peninsula eastward, 8 areas, including the offshore area of the Gulf of Alaska, yielded *Bucephaloides*. In 7 of the areas it was present in more than 10% of the sample (12% to 52%), but in the Fraser River sample it occurred only in 4%.

(5) *Tubulovesicula lindbergi* (Fig. 12). As in sockeye, this trematode also appears to be characteristic of eastern stocks. It was not found in the samples from Attu to the Sea of Okhotsk, but was recovered from 8 of the 9 areas, including the offshore sample in the Gulf of Alaska, in from 4 to 92% of the sample.

There was a progressive increase eastward and south from the Alaska Peninsula.

(6) *Lecithaster gibbosus* (Fig. 12). The incidence and intensity of infection in pinks from North American coastal areas was immensely greater than that in western high-seas areas. However, the Gulf of Alaska sample of pinks showed an infection rate similar to that of western high-seas areas. The apparent east-west differences are probably in reality a reflection of onshore-offshore differences, as noted in sockeyes.

(7) *Derogenes varicus* (Fig. 12). This stomach trematode was absent from the 5 samples from Attu westward and was found in 6 of the 9 eastern samples. The incidence varied from 8% to 21%. Since the parasite was absent from the one offshore area from which pinks were available, *Derogenes* may also be reflecting onshore-offshore rather than an east-west difference.

(8) *Eubothrium* sp. (Fig. 12). This cestode was very abundant in eastern coastal areas and found only in small numbers in 2 of the 4 areas west of Attu. However, the Gulf of Alaska offshore sample displayed an abundance of *Eubothrium* only slightly higher than that in the two western areas. Again *Eubothrium* may be reflecting offshore-onshore differences rather than east-west differences.

Summary. The differences in parasitism between eastern and western samples of pinks and sockeyes indicate that the study of parasites has promise as a means of distinguishing salmon stocks of North American and Asiatic origin. Of the parasites that were selected as possibly possessing value in distinction of stocks from the two continents, some indicated that Attu salmon are similar to samples to the west, whereas others indicated that Attu fish were similar to eastern samples. Completion of examinations of the 1956 collection of sockeyes and pinks should shed more light on the problem.

In the coastal areas of North America, several species of parasites increase or decrease in abundance from north to south. In both pinks and sockeyes, *Brachyphallus crenatus* (Trematoda), *Phyllobothrium caudatum* (Cestoda) and *Bolbosoma* sp. (Acanthocephala) tend to decrease in abundance from Alaska to the Columbia River or Puget Sound. On the other hand, in pinks, *Hemiurus levinseni, Tubulovesicula lindbergi* and *Eubothrium* sp. increase in abundance from north to south.

There is also evidence that some parasites are more prevalent in coastal areas. *Brachyphallus crenatus* and *Lecithaster gibbosus* in pinks and sockeyes appeared in greater numbers in coastal samples. Information to be obtained from 1956 samples may indicate other parasites that should be included in this category.

There are many differences between parasitism in sockeyes and pinks. Even the distribution and abundance of the same species of parasite may differ greatly between the two species of salmon. *Hemiurus levinseni,* for example, seems to indicate sockeyes of western origin, but in pinks it is common on both sides of the ocean. Some of the parasite differences are attributable to the longer fresh-water period of residence of sockeye before migrating to sea whereas others seem to reflect differences in feeding habits at sea.

There are some parasites of marine origin that are very restricted in distribution and may be useful in identifying the origin of fish on the high seas. The trematode *Podocotyle shawi* was found only in sockeyes from the Columbia River and the cestode *Diplocotyle* sp. was found in pinks and sockeyes only at Attu. If fish on the high seas are found to be infected with either of these parasites, their origin could be stated with considerable certainty.

A detailed report of the results and interpretations thereof obtained from samples collected in 1955 was distributed at the Commission's meetings in Seattle in November, 1955.

5. Use of Salmon Scales to Distinguish Stocks.

Canadian work in this field is at too early a stage to report results. Scale samples are being collected and exchanged with United States and Japanese agencies in order to compare and improve interpretations. It is intended to continue and intensify studies in this field in close co-operation with those of the other two member nations.

6. Oceanography.

Canadian oceanographic explorations in the northeast Pacific have provided five bodies of data.

(1) During the summer of 1936 the area 100 miles seaward of Vancouver Island was surveyed and studied. (2) A series of surveys of the Canadian offshore waters (east of longitude 141°W.) was made in winter, spring, and summer in 1950-51-52. (3) The co-operative project NORPAC covered the whole ocean north of latitude 22°N. during August, 1955. In this, the Canadian contribution was the part from latitude 47°N. to 55°N. and westward to longitude 180°. (4) During August, 1956, a survey was carried out covering the Gulf of Alaska and the adjacent ocean southward to latitude 40°N. and westward to longitude 175°W. This was the most extensive and comprehensive of the Canadian surveys. It included observations of temperature, salinity, total and inorganic phosphates, nitrates, silicates, alkalinity and hydrogen ion (pH) at all depths to 1,500 meters. (5) Daily observations of sea-water temperature from the surface to 150 metres depth have been taken at Ocean Weather Station PAPA (latitude 50°N., longitude 145°W.) since 1950. This program was expanded in August, 1956, to include salinity, the dissolved nutrient and respiratory constituents, and plankton.

Some results of preliminary analyses of recent data and comparisons with earlier data are summarized below.

Temperature. Both in August, 1955 (See INPFC Annual Report for 1955) and in August, 1956 (Fig. 13), the warmest waters appeared in mid-ocean south of latitude 40°N. From there to latitude 50°N. the temperatures decreased in a well-marked gradient to a region of minimum temperature, extending from the Aleutian Islands across the Gulf of Alaska. From this minimum, the temperatures increased shoreward all around the Gulf. In August, 1955, the isotherms extended almost east and west in mid-ocean. Closer to North America those isotherms south of latitude 47°N. veered southward, providing a marked temperature gradient toward the United States coast. Those isotherms north of latitude 47°N. veered northward and around the Gulf of Alaska. In August, 1956, the waters east of longitude 150°W. were warmer by one to two degrees than in the previous year, as indicated by the more northward slant of the isotherms.

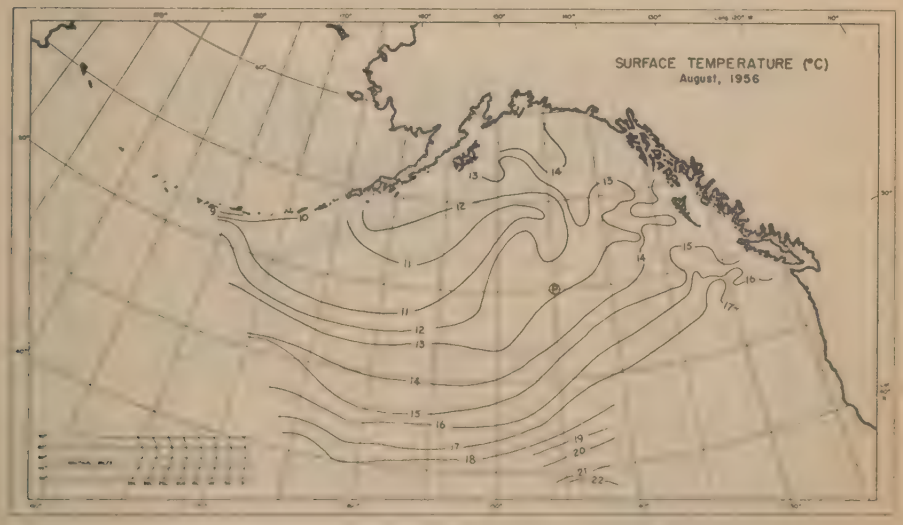

Fig. 13. Surface water temperatures (°C) in the northeast Pacific, August, 1956.

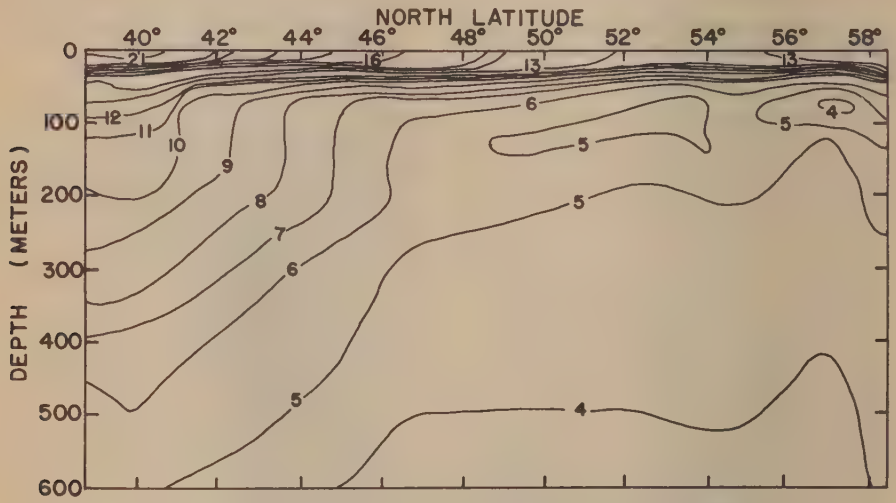

Fig. 14. Temperature (°C) structure along latitude 142°W., August, 1956.

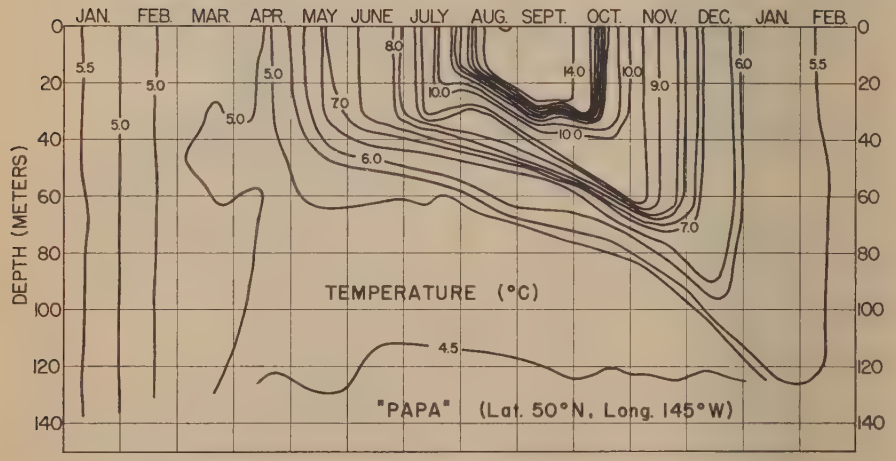
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB.
DEPTH (METERS)
0 20 40 60 80 100 120 140
5.5 5.0 5.0 5.0 5.0 7.0 8.0 10.0 14.0 10.0 9.0 6.0 5.5
6.0 10.0 7.0 4.5
TEMPERATURE (°C)
"PAPA" (Lat. 50°N. Long. 145°W)

Fig. 15. Sea water temperatures (°C), Station Papa, 1954-55.

The north-south sections (Fig. 14) show that there was a warm surface layer, separated from the deep cold waters by a marked thermocline between 25 and 50 metres depth. In the deep water, below 50 metres depth, the temperature decreased generally towards the north, and with depth. In both years there was evidence of temperature inversions at about 100 metres depth, but these were most apparent in 1956.

These diagrams were compared with mean August surface temperatures from pilot charts and other sources. The major features shown here are representative but the surface waters were slightly colder than average in 1955, and somewhat warmer than average in 1956.

A typical annual cycle of temperature at Ocean Weather Station PAPA (latitude 50°N., longitude 145°W.) is illustrated in Figure 15. In the winter the waters in the upper 100 metres of depth are isothermal. Vernal heating starts in April and continues until the end of September. During this time an upper zone of warmed water is created whose depth increases from 50 to 85 metres. The autumn cooling creates an isothermal layer which cools and deepens from late September to January. This cycle of heating and cooling in the upper 150 metres is apparent at least as far south as latitude 35°. Recent studies, however, indicate that south of latitude 40°N. the surface waters are always warmer than the deep waters. Northward from latitude 47° (surface isotherm 13° C. in Fig. 13) the surface waters become colder than the deep waters in February. Between these is a transition region where the extreme effect of winter cooling makes the water isothermal.

Below 150 metres existing evidence indicates that the temperature structure is nearly constant throughout the year and the small variations non-seasonal.

Salinity. The surface salinity in both years (Figures 16 and 17) was lowest along the North American coast, and increased towards mid-ocean and the Tropics. The surface gradient was most marked in mid-ocean between latitudes 40°N. and 47°N. and in the immediate vicinity of the coast. In the central part of the Gulf of Alaska, the surface salinity varied only slightly with a secondary maximum ($32.8\,^{0}/_{00}$) in the middle. Comparison of the

Fig. 16. Surface salinities($^{0}/_{00}$) in the northeast Pacific, July - August, 1955.

two surface charts shows that the general features of salinity distribution were similar in the two years except that the secondary maximum was farther north and more extensive in 1956.

In the Commission's Annual Report for 1955 (Fig. 3, p. 35) it was pointed out that there is an upper zone of low salinity water (generally less than 33.2 $^0/_{00}$) in the area north of the surface salinity gradient noted above. This was observed again in August 1956. A review of the more limited earlier data indicates that it is a permanent year-round feature distinguishing these from the more southern waters in which salinity is greatest at the surface.

"Sub-Tropic" and "Sub-Arctic" regions. Thus, in the upper 200 metres of depth in the North Pacific there are two distinct regions which may be designated as sub-tropic and sub-Arctic. The sub-Tropic is the region where the summer surface temperatures are greater than 21° C. and the surface salinity is greater than 34.2 $^0/_{00}$. Here the upper waters are always warmer than the deep waters, and the salinity decreases with depth. The sub-Arctic is the region where the summer surface temperatures are less than 20° C. and the surface salinity is less than 33.8 $^0/_{00}$. Here the upper waters become as cold as, or colder than, the deep water during the winter, the salinity increases with depth, and there is a well-defined upper zone of low-salinity water overlying the deep water.

Salmon fishing in the eastern Pacific shows that salmon occur in all coastal waters from California northward around the Gulf of Alaska, along the Aleutian Islands, and in the Bering Sea. Recent fishing for salmon and tuna on the high seas has found salmon generally scattered in the sub-Arctic waters as far south as latitude 42°N., but not in the sub-Tropic waters.

Circulation. In August, 1955, a broad slow movement (1 to 2 miles a day) entered the area from the west and progressed eastward toward the North American coast. This may be generally identified as the West Wind Drift, grading from Oyashio water along the northern side (latitude 50°N.) to Kuroshio water along the southern side (latitude 30°N.). Between longitudes 150°W. and 140°W. the waters north of latitude 47°N. veered northward

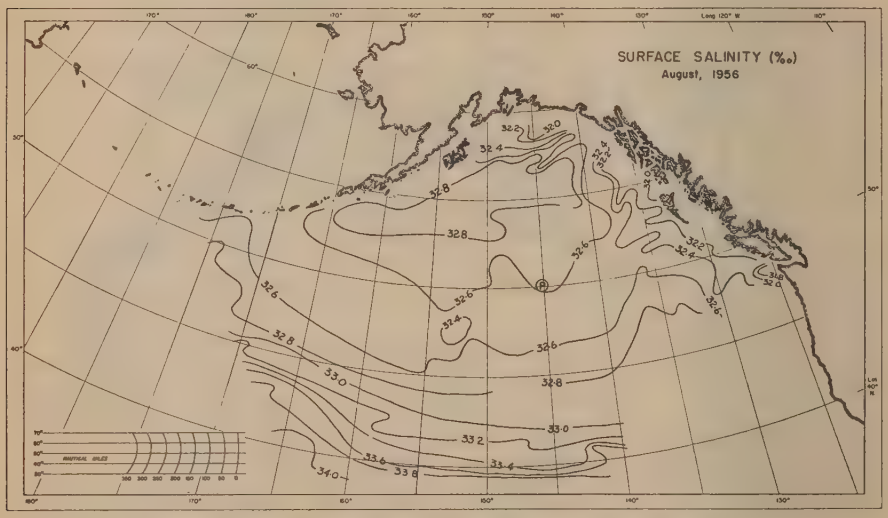

Fig. 17. Surface salinities($^0/_{00}$) in the northeast Pacific, August, 1956.

and circulated around the Gulf of Alaska in an anti-clockwise direction. These waters were apparently dissipated, to some degree, through the Aleutian Islands into the Bering Sea. A similar circulation was observed in August, 1956.

The rate of this circulation, as calculated from the observations in August, 1955 and 1956, was too slow to accord with other evidence, and it is suggested that the circulation is more rapid in winter. Further information will be available from returns of drift bottles released in August 1956 and at regular intervals from ocean weathership PAPA (50°N., 145°W.) and from the results of oceanographic surveys of this area in winter.

Plankton. Collections of plankton were made in association with the sampling of salmon on the high seas (see Section 1 above). Vertical hauls from 150 metres were made at the start and completion of each gill-net set and every six hours while running between stations. The gear used (45 cm. diameter net of 0.33 mm. mesh opening) was that recommended as a standard by the February 1956 conference on NORPAC results at Honolulu. Surface horizontal tows with the same net were also made at each fishing station for comparison with salmon stomach contents.

The wet weight of the plankton in each sample (including the separate wet weight of each group of organisms constituting more than 1% of the total) will be made available shortly in a data report with associated physical and chemical oceanographic data.

Crustacea usually amounted to 90% or more of the total sample; phyto-plankton, tunicates and medusae and, to a lesser extent, gastropods and squids, constituted most of the remainder. The survey area was covered three times (May-June, July and August) and great seasonal changes in the abundance and distribution of the plankton overshadowed geographical differences. In general the crustacean plankton increased from June to July and decreased again in August, although exceptionally rich samples were found about 155°W. longitude and 50°N. latitude. There are also some major geographical differences both in quantity and nature of the plankton (for example, the samples south of 43°N. were distinguished by the presence of goose-barnacle larvae *(Lepas)* but it is too early to generalize regarding the pattern of plankton distribution in relation to the physico-chemical oceanography or the salmon.

III. The Research Program of the North Pacific Fisheries Commission

A. PROGRESS IN 1957 IN CANADIAN RESEARCH ON PROBLEMS RAISED BY THE PROTOCOL

Canada's part in 1957 in the Commission's program of research on problems raised by the Protocol includes: (1) high-seas fishing to study salmon distribution and obtain samples; (2) studies of skeletal anatomy of salmon, of the parasites of sockeye and pink salmon and of salmon scales, to distinguish stocks of various river origins and discover their distribution on the high seas; and (3) study of the oceanography of the North Pacific to provide background for understanding salmon movements, and distribution. Canada also co-operated in other phases of the Commission's research program by provision of material for study by the United States and Japan and by efforts to recover tags placed on salmon on the high seas. Special fishing and tagging of young salmon leaving their rivers of origin was discontinued pending returns which will indicate the effectiveness of the tagging of the small fish.

1. HIGH-SEAS FISHING TO STUDY DISTRIBUTION AND OBTAIN SAMPLES

In 1957 the chartered vessels *Key West II* and *Fort Ross* carried out gill-net fishing in the North Pacific between May 13 and August 28. In accordance with plans developed at the March, 1957, meeting of the Committee on Biology and Research at Tokyo, fewer stations were occupied than in 1956 but more intensive fishing was carried out at each in order to obtain larger samples. Fishing was concentrated at or near seven primary stations in the Gulf of Alaska west to 155° W. Fishing was also done in the late spring at ten secondary stations off the United States coast south to about 43° N.; in the late summer at seven stations just north of the three southernmost primary stations; and at four stations closer to the North American coast between Latitudes 48° - 49° N. (See Figure 1).

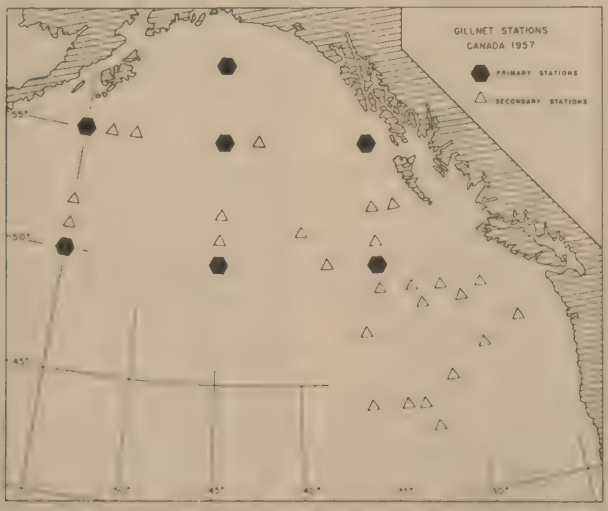

Fig. 1. Gillnet stations occupied by Canadian research vessels, 1957.

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Gear. Nylon surface gill-nets of four different mesh sizes were used at all stations, each net or "shackle" being about 50 fathoms long and 20 feet deep. In 1957 a ratio of three shackles of 4½" mesh to one each of 2½", 3¼" and 5¼" was used. In 1956 three shackles of 5¼" mesh were used to one each of 2½", 3¼" and 4½". In 1957 the nets were "hung in" 57% on the cork lines; in 1956, 50%. In 1957 more shackles were set at a time than in 1956, the maximum number being 41 as compared with 24.

At certain stations occupied by the *Key West II*, sets were also made with a sunken gill-net. This consisted of 4½" mesh webbing hung in the same manner as the surface nets but having a depth of about 40 feet. It was suspended on drop lines from a surface rope which was supported by 21 inflated plastic buoys. The sunken net could be set to operate at depths of 20 to 60 feet, 40 to 80 feet or 60 to 100 feet. As originally made up and used, in June and July, the net was 200 fathoms long. It was subsequently damaged and reduced to a length of 50 fathoms.

SALMON CATCHES IN THE NORTHEAST PACIFIC BY CANADIAN RESEARCH VESSELS IN 1957.

Date	Position		Gill-Net		Surface		Species					
	Lat. (°N.)	Long. (°W.)	Type	No. of Shackles	Depth (ft.)	Temp. (°C.)	Sockeye	Pink	Chum	Coho	Chi-nook	Total
KEY WEST II												
May 13	47° 39′	126° 46′	Surface	21	0-20	11.3	—	—	—	2	1	3
14*	46° 12′	129° 20′	Surface	21	0-20	11.3	—	—	—	—	—	—
15	46° 12′	129° 20′	Surface	21	0-20	11.1	—	2	1	—	—	3
16	45° 00′	131° 15′	Surface	21	0-20	11.4	—	—	—	—	—	—
17	43° 54′	133° 13′	Surface	21	0-20	11.3	—	—	—	—	—	—
18	44° 04′	134° 40′	Surface	17	0-20	10.4	—			—	—	—
26	44° 56′	133° 38′	Surface	17	0-20	11.9	—	—	—	1	—	1
27	44° 53′	136° 10′	Surface	17	0-20	11.5	—	—	—	—	—	—
28	47° 00′	136° 07′	Surface	17	0-20	10.7	—	3	1	—	—	4
29	48° 00′	133° 12′	Surface	17	0-20	10.8	1	—	1	—	—	2
30	48° 12′	130° 19′	Surface	17	0-20	11.9	—	—	—	—	—	—
June 14	54° 53′	134° 22′	Surface	36	0-20	10.5	20	12	209	22	—	263
14	54° 53′	134° 22′	Sunken		20-60	10.5	1	—	4	—	—	5
15	55° 00′	135° 00′	Surface	36	0-20	10.5	38	23	353	25	—	439
29	55° 00′	145° 00′	Surface	36	0-20	10.5	11	9	80	17	1	118
29	55° 00′	145° 00′	Sunken		20-60	10.5	2	7	51	1	—	61
July 1	55° 00′	153° 17′	Surface	36	0-20	10.5	39	11	24	2	—	76
1	55° 00′	153° 17′	Sunken		20-60	10.5	9	8	33	—	—	50
2*	55° 00′	155° 00′	Sunken		40-80	10.0	15	1	5	—	—	21
3	55° 00′	155° 00′	Surface	36	0-20	10.0	125	9	64	—	—	198
3	55° 00′	155° 00′	Sunken		40-80	10.0	20	5	46	1	—	72
8	58° 00′	145° 00′	Surface	36	0-20	13.3	59	9	21	—	—	89
8	58° 00′	145° 00′	Sunken		20-60	13.3	10	7	22	6	—	45
9	58° 00′	145° 00′	Surface	36	0-20	13.0	18	19	71	12	—	120
9	58° 00′	145° 00′	Sunken		40-80	13.0	4	7	24	1	—	36
9*	58° 00′	145° 00′	Surface	4	0-20	13.0	—	1	1	—	—	2
9*	58° 00′	145° 00′	Sunken		40-80	13.0	—	1	1	—	—	2
10	58° 00′	145° 00′	Surface	35	0-20	13.0	37	24	36	8	—	105
10	58° 00′	145° 00′	Sunken		60-100	13.0	—	5	4	—	—	9
11*	55° 00′	145° 00′	Surface	6	0-20	12.5	—	—	1	—	—	1
11	55° 00′	145° 00′	Sunken		40-80	12.5	1	—	—	—	—	1
12	55° 00′	145° 00′	Surface	35	0-20	12.5	7	117	25	5	—	154
12	55° 00′	145° 00′	Sunken		40-80	12.5	2	9	—	—	—	11
31	55° 00′	135° 00′	Surface	41	0-20	15.2	17	45	3	2	—	67
31	55° 00′	135° 00′	Sunken		20-60	15.2	1	4	—	—	—	5
Aug. 1	55° 00′	135° 00′	Surface	41	0-20	15.2	15	56	3	3	—	77
1	55° 00′	135° 00′	Sunken		40-80	15.2	—	—	—	—	—	—
3	55° 07′	142° 40′	Surface	41	0-20	14.8	1	1	16	—	—	18
3	55° 07′	142° 40′	Sunken		20-60	14.8	—	—	—	—	—	—
8	55° 00′	135° 00′	Surface	41	0-20	14.0	2	5	—	—	—	7
8	55° 00′	135° 00′	Sunken		20-60	14.0	—	—	—	—	—	—
16	55° 00′	145° 00′	Surface	41	0-20	14.5	2	—	19	—	—	21
16	55° 00′	145° 00′	Sunken		20-60	14.5	—	—	2	—	—	2
18	55° 03′	151° 30′	Surface	38	0-20	15.0	11	—	71	—	—	82
18	55° 03′	151° 30′	Sunken		20-60	15.0	1	—	4	—	1	6
19	55° 00′	152° 56′	Surface	38	0-20	14.4	16	—	133	—	—	149
19	55° 00′	152° 56′	Sunken		20-60	14.4	—	—	2	—	—	2
22	58° 00′	145° 00′	Surface	38	0-20	15.5	2	1	128	—	—	131

* Day set. All others night sets.

Date	Position Lat. (°N.)	Position Long. (°W.)	Gill-Net Type	Gill-Net No. of Shackles	Gill-Net Depth (ft.)	Surface Temp. (°C.)	Species Sockeye	Pink	Chum	Coho	Chinook	Total
FORT ROSS												
May 19	55° 00′	135° 00′	Surface	20	0-20	8.2	3	—	5	—	—	8
20*	52° 30′	135° 00′	Surface	20	0-20	9.0	—	—	1	1	—	2
21	52° 30′	135° 00′	Surface	20	0-20	9.0	—	—	—	—	—	—
22*	55° 00′	135° 00′	Surface	20	0-20	9.0	1	—	1	—	—	2
23	55° 00′	135° 00′	Surface	20	0-20	8.2	2	—	58	2	—	62
June 8	50° 00′	135° 00′	Surface	35	0-20	13.8	30	18	46	115	—	209
9*	50° 00′	135° 00′	Surface	16	0-20	13.8	1	—	—	—	—	1
9	50° 00′	135° 00′	Surface	34	0-20	13.8	54	12	36	48	—	150
10	50° 00′	135° 00′	Surface	33	0-20	12.9	63	6	32	23	—	124
14	50° 00′	145° 00′	Surface	33	0-20	9.0	38	17	12	24	—	91
16	50° 00′	145° 00′	Surface	33	0-20		Species unidentified					
21	50° 00′	155° 00′	Surface	30	0-20	8.4	11	4	7	1	—	23
22*	50° 00′	155° 00′	Surface	30	0-20	8.8	—	—	—	—	—	—
22	50° 00′	155° 00′	Surface	30	0-20	8.3	4	3	6	2	1	16
23	50° 00′	155° 00′	Surface	30	0-20	9.0	66	13	11	8	1	99
24	50° 00′	155° 00′	Surface	30	0-20	8.3	2	—	—	2	—	4
28	50° 00′	145° 00′	Surface	26	0-20	9.7	7	10	14	2	—	33
30	50° 00′	145° 00′	Surface	26	0-20	10.0	8	19	34	17	—	78
July 2	50° 00′	138° 08′	Surface	26	0-20	11.0	36	1	2	—	—	39
3	50° 00′	135° 00′	Surface	26	0-20	11.7	20	12	13	—	—	45
20	51° 00′	135° 00′	Surface	35	0-20	14.4	120	2	—	—	—	122
21	51° 00′	135° 00′	Surface	35	0-20	13.4	7	—	—	—	—	7
24	51° 00′	145° 00′	Surface	35	0-20	12.1	—	—	—	—	—	—
26	51° 00′	145° 00′	Surface	35	0-20	11.5	—	—	—	—	—	—
28	51° 00′	155° 00′	Surface	35	0-20	11.0	2	—	6	—	—	8
29	52° 00′	155° 00′	Surface	35	0-20	11.0	2	—	3	—	—	5
30	52° 00′	155° 00′	Surface	35	0-20	11.0	22	—	2	—	—	24
31	52° 00′	155° 00′	Surface	35	0-20	11.5	9	—	16	—	—	25
Aug. 2	52° 00′	145° 00′	Surface	35	0-20	13.0	—	—	11	—	—	11
4	52° 00′	145° 00′	Surface	35	0-20	13.0	3	1	14	1	—	19
6	52° 00′	135° 00′	Surface	35	0-20	13.7	—	—	5	1	—	6
7	52° 00′	135° 00′	Surface	35	0-20	13.7	—	—	1	—	—	1
8	52° 00′	134° 00′	Surface	35	0-20	14.5	—	—	2	—	—	2
18	52° 08′	133° 25′	Surface	35	0-20	15.1	—	—	—	—	—	—
19	52° 30′	135° 00′	Surface	35	0-20	15.9	—	—	—	—	—	—
22	51° 00′	140° 00′	Surface	35	0-20	14.4	—	—	—	—	—	—
25	49° 01′	134° 24′	Surface	35	0-20	15.4	—	—	—	—	—	—
26	48° 55′	132° 42′	Surface	35	0-20	15.8	—	—	—	—	—	—
27	48° 50′	131° 06′	Surface	35	0-20	15.7	—	—	—	—	—	—
28	48° 40′	129° 32′	Surface	35	0-20	16.7	—	—	—	—	—	—
Totals							998	519	1,797	355	5	3,674
							Species unidentified (June 16)					47
												3,721

* Day set. All others night sets.

Salmon catches and distribution. Preliminary examination of the surface gill-net catches (see accompanying table) suggests that, in general, catches of pink and chum salmon were relatively great in the northern part of the Gulf of Alaska, coho in the eastern part and sockeye south of the Alaskan Peninsula. As in 1956 the southern limit of the catches moved northward as the summer advanced. Catches were generally smaller in the latter part of the summer, apparently reflecting the migration of mature salmon from the high seas towards the spawning grounds. In the total catch for the season, sockeye and chums showed a percentage increase over 1956, while pinks and cohos formed a relatively smaller percentage of the catch. The comparison is shown by the following figures:

		Sockeye	*Pink*	*Chum*	*Coho*
1956	%	21	27	37	15
1957	%	27	14	49	10

The catches of the sunken gill-net were sometimes larger, per unit area of net, than the catches of surface nets of the same mesh, fished at the same time. Operation of this net was only practicable in good weather. Sockeye were mainly caught close to the surface, but pinks and chums were on some occasions more numerous in the sunken net. Both these species

were taken down to the deepest level (60 to 100 feet) at which fishing was conducted. The data obtained are too limited to justify firm conclusions, but they raise the question of how representative the surface catches are. Further investigation of the vertical distribution of Pacific salmon seems desirable.

Associated studies. Oceanographic observations and plankton collections were made in conjunction with the high-seas fishing in 1957 as in 1956. In the Gulf of Alaska, surface waters were warmer and plankton sparser in 1957 than in 1956.

2. SKELETAL ANATOMY OF PACIFIC SALMON

In 1957 the descriptions of the osteology of the five principal species of Pacific salmon of the genus *Oncorhynchus* at various stages of their life history have progressed far enough to make it possible to distinguish between the changes associated with the approach of maturity and the more stable differences which might be used to distinguish stocks of different origins. The search for such differences is still in progress and offers limited promise.

3. STUDY OF SALMON PARASITES TO DISTINGUISH STOCKS

A. Results of examination of 1956 samples of sockeye and pink salmon

From 1956 collections 1,391 sockeye (400 downstream migrants and 991 adults or high-seas specimens) and 777 pinks (668 adults and 109 juveniles) were examined. As in 1955 collections, a sample of 25 fish was examined from each locality, where possible. Pink fry were not examined because the 1955 samples had indicated that they were free or almost free of parasites.

The sockeye smolts were taken from 16 North American localities from the Columbia River to Bristol Bay. Sockeye adults were examined from 21 localities along the North American coast and the Aleutians and from 35 localities on the high seas. The latter were spaced approximately at intervals of 5 degrees of longitude across the North Pacific from 140° W. to the Sea of Okhotsk and between 165° W. and 180° in the Bering Sea. Most adult coastal and high-seas sockeye were caught in late June, July and August with a few in early June and early September.

Juvenile pink salmon were taken in the vicinity of the outflow of the Skeena River, British Columbia, in July and August. Adult pinks came from 15 localities along the North American coast and the Aleutians, one in Hokkaido, Japan, and 29 on the high seas distributed as in the case of sockeye, but with less complete coverage. Lack of adequate samples between 160° W. and 180° is particularly noticeable. In the Bering Sea, samples were taken only at 170° W and 180°. Most of the high-seas samples were taken in June and July, with some in May and August. The coastal samples were taken mainly in July and August with some in September and early October.

About 50 species of parasites were found, about one-third of which were of freshwater origin. In sockeye salmon 6 species (2 from fresh water), and in pinks 3 species, appear to be significant in differentiating between Asiatic and North American stocks of salmon. It is well to be reminded that the data apply only to the distribution of various stocks when the samples were taken and may not reflect their distribution at other times of the year or in other years.

(i) **Sockeye salmon**

(a) **Parasites acquired in the sea.** Three of the parasites which salmon acquire in the sea *(Hemiurus, Tubulovesicula* and *Echinorhynchus)* were more or less restricted in high-seas samples either to the eastern or to the western Pacific; a fourth *(Anisakis),* although present in almost every fish, had a much higher intensity in western than in eastern samples. The parasite characteristics of eastern high-seas samples are similar to those of North American coastal samples, and it appears that the continental origin of sockeye stocks may be traced by use of the parasites discussed below.

Hemiurus levinseni was present in the 5 samples from 171° E. to the Sea of Okhotsk. It was absent from 6 Bering Sea samples east of 180°, from 8 Pacific samples between 172° E. and 155° W., and from a sample at 140° W. It was present but rare at 145° W. and 150° W. In coastal sockeyes, *Hemiurus* was encountered in the adjacent areas of Petersburg, southeast Alaska, and the Skeena River, British Columbia, and a single specimen in one fish at Kuskokwim, western Alaska. In relating the distribution of *Hemiurus* in high-seas sockeyes to that in coastal sockeyes, it seems that the presence of *Hemiurus* at 145° W. and 150° W. indicates origin of at least some of these fish from the Petersburg-Skeena area. Absence of this trematode at 140° W. can be related to its absence in the more southerly North American coastal samples. The complete lack of *Hemiurus* in all high-seas samples between 172° E. and 155° W. and in the eastern Bering Sea parallels its absence in all Alaskan areas north of Petersburg, except for the single occurrence at Kuskokwim. The consistent presence of *Hemiurus* between 171° E. and the Okhotsk Sea probably indicates the Asiatic origin of these samples.

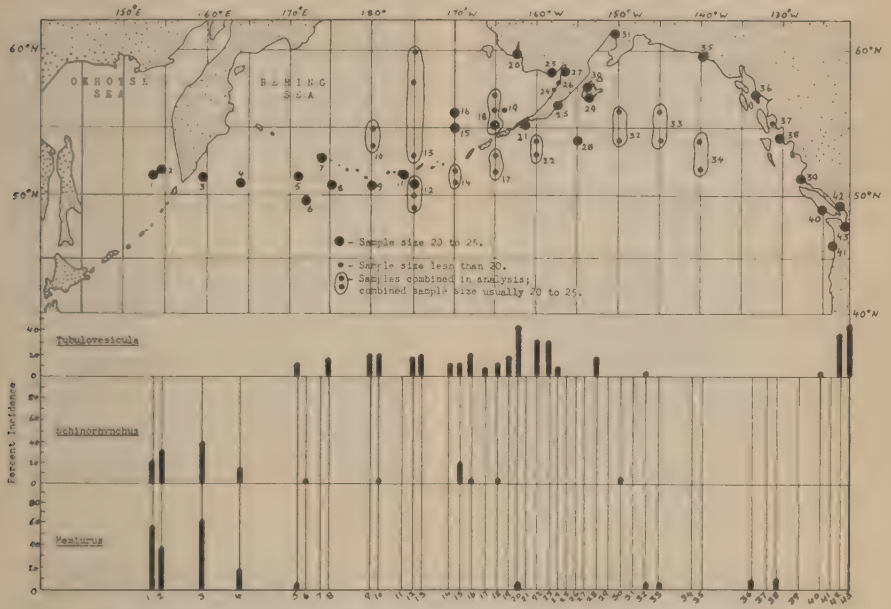

Fig. 2. Distribution of sockeye salmon samples examined for parasites from 1956 collections, and incidence, in percent, of *Hemiurus, Echinorhynchus* and *Tubulovesicula* in these samples.

Tubulovesicula lindbergi was well represented in all high-seas samples from 150° W. to 175° E. and at 171° E., but lacking at 172° E. and from 164°E. to the Okhotsk Sea. It was present in all Bering Sea samples from 180° to 165° W. In coastal North American samples it was present in 3 Bristol Bay or western Alaskan areas and in 3 southerly areas in British Columbia and Puget Sound. Although many of the North American coastal samples had no *Tubulovesicula* in 1956, the samples from some of these areas showed it in 1955. Its presence in the eastern Bering Sea samples and in high-seas areas, south of the Aleutians, between 175° E. and 150° W., and at 171°E., probably relates many of these fish to North American coastal samples. The sample at 172° E. is related to those taken between 164° E. and the Okhotsk Sea, by the absence of *Tubulovesicula*.

The *Tubulovesicula* taken in the southerly coastal areas of North America were immature, whereas they were mature in all other coastal areas and high-seas samples. It appears that *Tubulovesicula* in southern North American coastal areas is the result of infection upon re-entry into coastal waters and that fish from these areas may not be related to those infected with *Tubulovesicula* on the high seas. It is probably significant that at 140° W. and 145° W., *Tubulovesicula* was not found in the samples and that these fish seem to represent stocks from southern North American coastal areas.

At 171° E. where both *Hemiurus* and *Tubulovesicula* were found, they occurred in different fish in the sample, which is compatible with the interpretation that in this area *Hemiurus* indicates Asiatic origin and *Tubulovesicula* indicates North American origin.

Echinorhynchus gadi was noted in 5 of the 6 samples from 172° E. to the Okhotsk Sea. It was absent in high-seas samples south of the Aleutians at 171° E. and from 175° E. to 140° W. In the Bering Sea it occurred in 4 of 6 samples between 180° and 165° W., but not in the sample at 175° W. nor in one of the 2 samples at 165° W. In all North American coastal samples it was seen only in one fish from Kodiak. Its absence from 175° E. to 140° W. south of the Aleutians suggests that these fish are of North American origin. From 172° E. to the Okhotsk Sea, the occurrence of *Echinorhynchus*, except at 171° E., suggests a predomin-

ance of Asiatic stocks. In the Bering Sea its presence from 165° W. to 180° implies that some of these fish are of Asiatic origin. They might be fish of North American origin which had been in Asian waters, but *Echinorhynchus* and *Tubulovesicula* (which probably indicates North American origin) never occurred in the same individual fish (although in the same sample).

The distribution and incidence of *Hemiurus*, *Tubulovesicula* and *Echinorhynchus* in sockeyes is shown graphically in Figure 2.

Anisakis larvae are known to accumulate as the fish grow older. Therefore any comparison of the levels of infection of various stocks must take into account the number of years the fish have spent at sea. Although almost 100% of the samples were infected with this parasite, there were pronounced differences in the level of infection between samples taken from 171° E. to the Sea of Okhotsk and almost all those taken east of 171°E., except for the Kuskokwim, Alaska, sample. The average level of infection of fish with 2+ and 3+ years at sea in the western group of samples was greater than 21 and 28, respectively, whereas for samples taken east of 171° E. the corresponding average numbers of *Anisakis* per fish were less than 21 and 28, and frequently only half of these values. Many more individual sockeye in the samples between 171° E. and the Okhotsk Sea than in the more easterly samples had more than 20 or 25 *Anisakis* in the 2+ and 3+ groups, respectively. It seems that high levels of *Anisakis* infection are generally indicative of Asiatic stocks.

(b) **Parasites acquired in fresh water.** *Triaenophorus crassus* larvae were found only in adults and smolts of the Wood River - Nushagak River systems and less commonly at Naknek River, both in Bristol Bay. Smolts from Asiatic areas were not available, but the literature does not record *T. crassus* as a parasite of sockeyes in Kamchatka, where studies on parasites of salmon have been carried out. *Triaenophorus* matures only in pike and hence can be present in sockeyes only if sockeye and pike occur in the same waters. *Triaenophorus* was found occasionally in 4 of the 8 high-seas samples between 150° W. and 175° E., south of the Aleutians. On the basis of our present knowledge it is concluded that *Triaenophorus*-infected sockeyes on the high seas are of Bristol Bay origin.

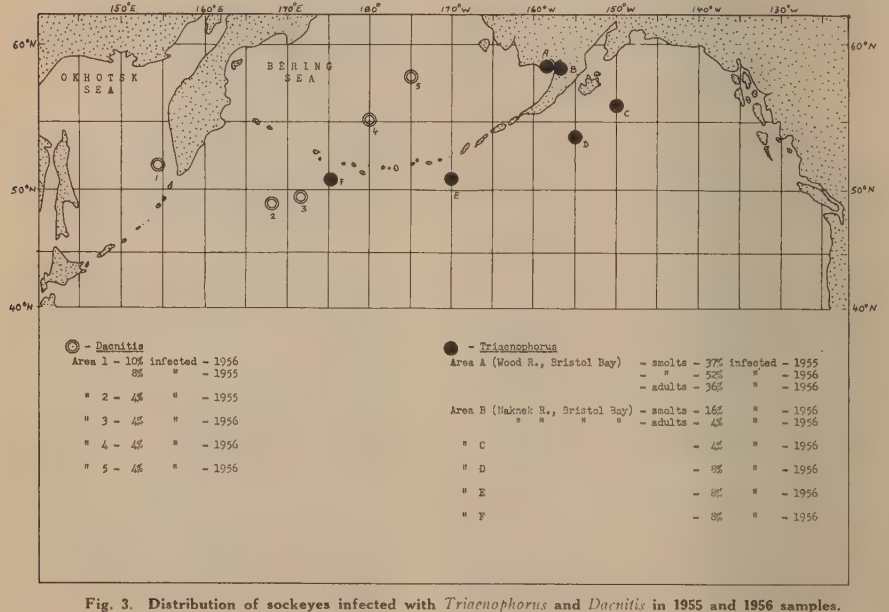

Fig. 3. Distribution of sockeyes infected with *Triaenophorus* and *Dacnitis* in 1955 and 1956 samples.

Dacnitis truttae was found occasionally in 3 of 6 samples from the Okhotsk Sea to 172° E., south of the Aleutians, and at 180° and 175° W. in the Bering Sea. The literature records it is a parasite of sockeyes in the Kamchatka Peninsula. All high-seas samples east of 172° E., south of the Aleutians and east of 170° W. in the Bering Sea, and all North American samples of adults and of smolts were free of this parasite. On the basis of present knowledge, it is concluded that *D. truttae* in high-seas sockeyes is indicative of their Asiatic origin.

Figure 3 shows the distribution and records the incidence of these two parasites in 1955 and 1956 samples of sockeyes.

(c) **Summary.** On the basis of the occurrence of three parasites acquired in the sea (*Hemiurus, Echinorhynchus* and *Tubulovesicula*), and of the intensity of infection by *Anisakis*, samples taken on the high seas east of 175° E., south of the Aleutians, showed a marked relationship to North American coastal samples. Samples from west of 171° E. appeared to be different from those taken in North American coastal waters and from those taken east of 175° E., and probably represent Asiatic stocks. Two samples, at 171° E. and 172° E., on the basis of the same parasites contain fish relating them to samples taken both east and west of these meridians. In the Bering Sea, on the other hand, *Echinorhynchus*, as well as *Tubulovesicula*, were present in samples between 165° W. and 180°. These two parasites never occurred in the same fish and if, as seems to be the case, *Echinorhynchus* in sockeyes indicates Asiatic origin and *Tubulovesicula* North American origin, then the Bering Sea samples represented fish from both continents. Furthermore, two parasites acquired in fresh water support the above implications. *Triacnophorus*, indicative of certain Bristol Bay stocks, was found occasionally in samples as far west as 175° E. and *Dacnitis*, indicative of Asian stocks, was found in samples as far east as 172° E., south of the Aleutians, and at 180° and 175° W. in the Bering Sea.

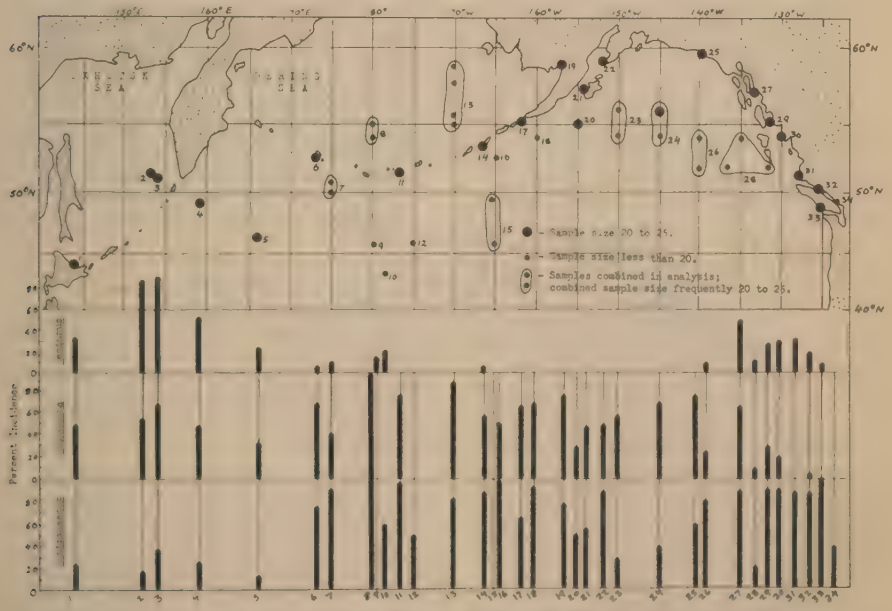
60°N
50°N
40°N
Percent Incidence
- Sample size 20 to 25.
- Sample size less than 20.
- Samples combined in analysis; combined sample size frequently 20 to 25.

Fig 4. Distribution of pink salmon samples examined for parasites from 1956 collections, and incidence, in percent, of *Hemiurus, Bolbosoma* and *Contracaecum* in these samples.

(ii) **Pink salmon**

No one species of parasite was found whose distribution permitted the division of high-seas samples into two groups, an eastern and a western, as was indicated for sockeyes. Nor was there a regular gradation in the incidence of certain parasites in an east-west or west-east direction, as appeared to be the case in the less adequate 1955 samples. Nevertheless there were certain marked discontinuities in the distribution or abundance of certain parasites which seem to indicate three main groups of high-seas samples which can be related to coastal samples from three regions. The parasites which best illustrate this division are *Hemiurus, Bolbosoma* and *Contracaecum.* The distribution and incidence of infection of pink salmon by these parasites is shown graphically in Figure 4.

Hemiurus levinseni occurred in high-seas samples from 140° W. to 132° W. and from the Okhotsk Sea to 178° W. From 175° W. to 145° W. and in the Bering Sea samples at 170° W. and 180° it was absent. *Hemiurus* was well represented in coastal samples from southeast Alaska, southward and from Hokkaido. It was also observed in one fish each at Unalaska and Attu. It was absent from central and western Alaska.

Bolbosoma was encountered in less than 25% of the fish in samples from 140° W. to 132° W. and in coastal samples south of Ketchikan in southeast Alaska. In central and western Alaska the incidence was about 50% to 75% in each sample, which coincides generally with that in high-seas samples from 145° W. to 165° W. and in the Bering Sea. At Hokkaido and from 175° E. to the Okhotsk Sea the incidence was approximately 50%.

A high incidence of *Contracaecum,* between 50% and 100%, characterized samples taken in North American coastal waters and generally in high-seas samples east of 175° E. and in the Bering Sea. The incidence of *Contracaecum* in samples from west of 175° E. on the high seas and at Hokkaido was about 25%. Not only was the incidence higher in eastern samples, but the intensity of the infection was markedly greater.

On the basis of these three parasites the following groups of samples have been recognized:

(1) **Asiatic group.** This group is characterized by the prominence of *Hemiurus* and *Bolbosoma.* It includes the samples from Hokkaido and from the Sea of Okhotsk to 175° E. at approximately 50° N., intergrading between 170° E. and 175° E. with group (3).

(2) **Southern North American group.** This group is characterized by the prominence of *Contracaecum* and *Hemiurus,* with low incidence or absence of *Bolbosoma.* It includes coastal samples from southeast Alaska southward and high-seas samples west to 140° W. Another parasite, *Parahemiurus,* also is restricted to this group. From south to northwest in this group there is an increase in *Bolbosoma* incidence, providing a transition to group (3).

(3) **Central and western Alaska group.** The dominant features of this large group of samples are the almost complete absence of *Hemiurus* and the prominence of *Contracaecum* and *Bolbosoma.* It includes coastal samples from central and western Alaska and high-seas samples between 145° W. and 165° W. at latitudes north of 50° N. The samples at 175° E. and at Attu show the high *Contracaecum* of this group, but also the *Hemiurus* of group (1). There thus appears to be an overlap with the Asiatic group between 170° E. and 175° E.

A small group of samples taken between 165° W. and 180° at latitudes south of 50° N. (principally between 42° N. and 46° N.) did not possess the combinations of characteristics of any of the above 3 groups, mainly because of the complete lack of *Bolbosoma.* Two samples taken between 178° W. and 180° showed the *Hemiurus* character of the Asiatic group, the sample at 178° W. showing also the high *Contracaecum,* similar to the North American group. At 175° W. the high *Contracaecum* (50%) and lack of *Hemiurus* showed possible relationship with North American groups. A sample at 165° W. was even more difficult to place in any of the 3 groups, because of a general lack of parasites, but the high incidence of another trematode, *Bucephaloides,* tends to show relationships with North American samples.

B. **Comparison with 1955 samples**

The number of samples of adult or high-seas sockeyes and pinks examined from 1956 catches was more than twice the number examined from 1955 catches. This was due largely to a much more extensive distribution of high-seas samples, but also a greater coverage of coastal areas. Sockeye smolts from more localities were also examined in 1956.

There is a reasonably good similarity in incidence of parasites and degree of infection in the localities from which fish were examined in both years. Certain rare parasites did not occur in both years, other parasites showed a lower incidence or intensity in 1956, and still others seemed to occur more frequently. No attempt has been made, however, to test for statistical significance of these differences. The parasites acquired in the sea which apparently show continental origins

of sockeyes were very similar in the two years. The role of *Lecithaster* in identifying eastern stocks was considered a possibility in 1955 samples but it has since been noted that *Lecithaster* probably reflects differences between coastal and high-seas samples rather than east-west differences. The significance of *Anisakis* was not appreciated in the 1955 samples, because the sockeyes had not been aged. The significance of parasites acquired in fresh water, such as *Triaenophorus* and *Dacnitis*,

Fig. 5. Oceanographic stations occupied by Canadian vessels in February and August, 1957.

in identifying the continental origin of individual fish, was not fully appreciated in 1955 because of the paucity of high-seas samples.

In the summary prepared last year, on the basis of 1955 samples, 8 parasites were selected as possibly significant in the identification of two groups of high-seas pink salmon samples relating to their continental origin. These were *Bolbosoma, Contracaecum, Echinorhynchus, Bucephaloides, Tubulovesicula, Lecithaster, Derogenes* and *Eubothrium. Bolbosoma* and *Contracaecum* were considered of prime importance, as again noted this year, although 3 groups, rather than 2 groups of samples, are recognized. In addition, *Hemiurus* is considered of importance in 1956 samples, and basically showed the same type of distribution in 1955 samples, but was not then considered in the distinction of stocks because of the prime concern with searching for two groups of stocks, rather than three, as conceived at present. *Lecithaster, Derogenes,* and *Eubothrium,* of doubtful value in 1955 samples, seem to show differences which reflect coastal in contrast to high-seas distribution, rather than east-west differences. *Bucephaloides, Tubulovesicula* and *Echinorhynchus* may still be of value, but their occurrence does not at present give a sufficiently clear picture to delimit groups of pink salmon stocks.

C. Examination of 1957 samples

Examination of samples collected in 1957 is now in progress. Samples are available from essentially the same localities as in 1956 but with more adequate samples of pinks from the high seas. Furthermore, at least two, and possibly three samples, taken at intervals of two weeks to one month from the same high-seas stations, are available in many instances. Examination of these samples should yield information on the constancy or variability of parasitism in samples taken at the same locality through the months of June to August.

4. OCEANOGRAPHY

Since the last summary report (September, 1956) two more surveys of the Gulf of Alaska and the adjacent ocean have been completed (Figure 5). The first, from January 22 to March 4, was the first truly winter survey of the sub-Arctic regions of the North Pacific. The second was made from July 22 to August 30, 1957, and covered the same area with a similar cruise plan. This was the fourth summer survey in this area. The previous surveys were: August, 1954, by the University of Washington; August, 1955, by the University of Washington and the Pacific Oceanographic Group (P.O.G.) of the Fisheries Research Board of Canada in the Nor-Pac project; and August, 1956, by P.O.G. Data records of the 1955, 1956 and 1957 surveys have been distributed, and it is expected that data records of the 1957 winter and summer surveys will be available before the 1957 meeting of the Commission.

Analysis of the data is proceeding. A progress report, similar to, but more advanced than, that made to the Committee on Biology and Research in March, 1957, is in preparation. In the meantime it is possible to summarize the observational work of the past year and comment on the more important features.

Temperature. Figure 6 shows the temperature distribution in the area during February, 1957. The temperature decreased from south to north and the isotherms sloped northeastward. The surveys only reached the northern fringes of the Polar Front. It is evident, however, that the warm southern water occurred farther north near the American continent than in mid-ocean. The coldest water was found in an elongated area a short distance south of Kodiak and the Shumigan Islands. This cold centre has the same location as the coldest water found in the summer surveys.

The cross-sections show that in the eastern part of the area (Sections A and B) the water was warmest at the surface (upper 10 to 20 meters) and became colder with depth to about 100 meters. Below this the temperature increased about one degree to 200 meters. Below 200 meters the temperature again decreased slightly with depth to 1,000 meters. Thus there is an upper zone separated from a deep zone by a positive thermocline. The temperature inversion in the positive thermocline is stable because it coincides with the halocline. At the western limit of the survey (Section C), the positive thermocline was only in evidence near the Alaska Peninsula. Farther offshore the waters were isothermal from the surface to about 200 meters depth. Below this the temperature decreased to 1,000 meters depth.

Figure 7 shows the grand monthly mean of the surface sea-water temperature at ocean weather station "PAPA" (50° N., 145° W.) from 1950 through 1956 and, for comparison, the monthly mean temperatures through the past winter (1957). It is evident that the surface waters were warmer than average, both at the surface and at 70 meters depth.

The temperature distribution observed in August, 1957, is shown in Figure 8. As usual the waters were warmest at the southern limits of the area, and coldest in the region just south of Kodiak and the Shumigan Islands. This cold centre was rather smaller, warmer, and farther

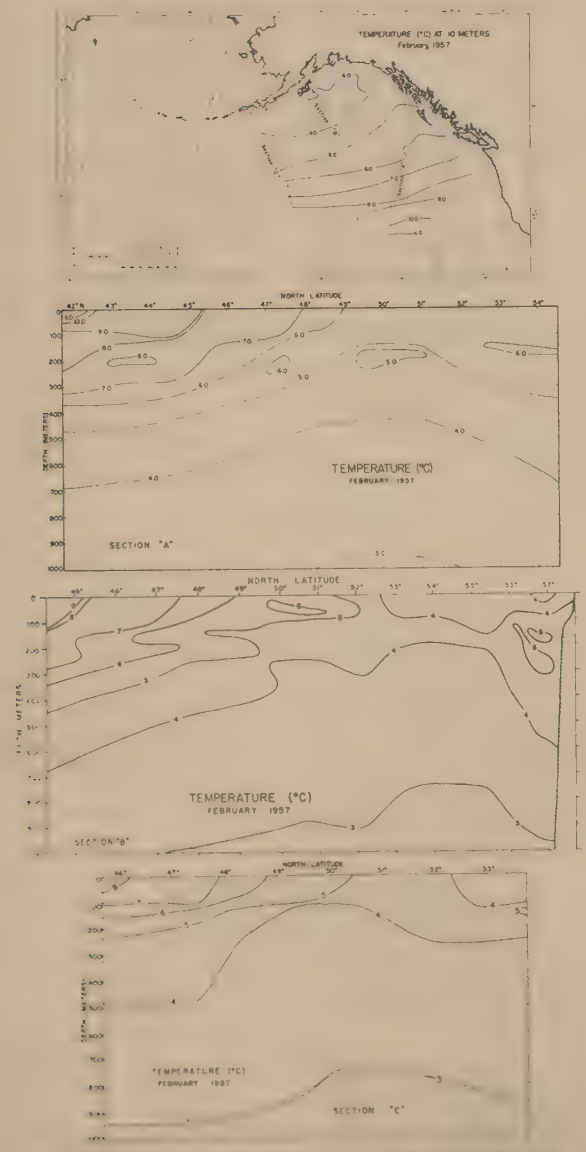

Fig. 6. Temperatures in the eastern North Pacific in February, 1957.

westward than in previous summers. The isotherms entered the area from the west and curved around the cold centre so that there was a warm area between station "PAPA" and the Canadian coast. A small body of warm surface water was isolated in the northeast part of the area near the Alaskan coast. This has not been observed in previous surveys. Further study of the data will be required to determine its origin and cause.

The cross-sections show that the warm upper waters extended to a thermocline between 30 and 100 meters depth as usual. Below this the waters became colder with depth. There was an evident dome of cold water in the deep zone, immediately below the cold centre off Kodiak and the Shumigan Islands. This dome was separated from the near surface waters by a temperature inversion in the halocline. This structure has been observed regularly in the summer surveys and is evidence that the cold dome and the deep waters are separated. In previous summer surveys temperature inversions in the halocline have been regularly observed in the eastern part of the area, between station "PAPA" and the Canadian coast, but this structure was not common in August, 1957.

Salinity. The winter and summer salinity data are summarized in Figures 9 and 10. Here it is evident that the distribution and structure were similar in the two seasons. There was an upper zone of salinity less than 32.8‰ extending to about 100 meters depth. From 100 to 200 meters depth there was a halocline in which the salinity increased one part per thousand to 33.8‰. Below this the salinity increased to about 34.4‰ at 1,000 meters. The density gradient inherent in the halocline makes the temperature inversions possible and stable in this zone.

The surface salinity was greatest at the southern limit of the area, became less towards Latitude 50° N. and towards the coast, all around the region. There was a small secondary maximum, greater than 32.8‰ in the vicinity of the cold centre near Kodiak and the Shumigan Islands.

The sections show that the halocline was continuous and at nearly the same depth over the whole area. It was shown in the NorPac (1955) data that this upper zone of low salinity extended over the sub-Arctic region where precipitation exceeds evaporation. It vanishes to the south at the "Polar Front" where the halocline comes to the surface. The surveys in 1957 did not reach far enough south to determine the limit of this structure. They approached it, however, as indicated by the increasing surface salinity at the southern boundary.

The secondary maximum at the cold centre is shown to be an upper zone phenomenon. The halocline (33.0 to 33.8‰) is continuous through this region. This is positive evidence of the separation of the upper and deep zones.

In the deep zone the salinity increased only slightly with depth, and there was little or no significant variation over the region or from winter to summer.

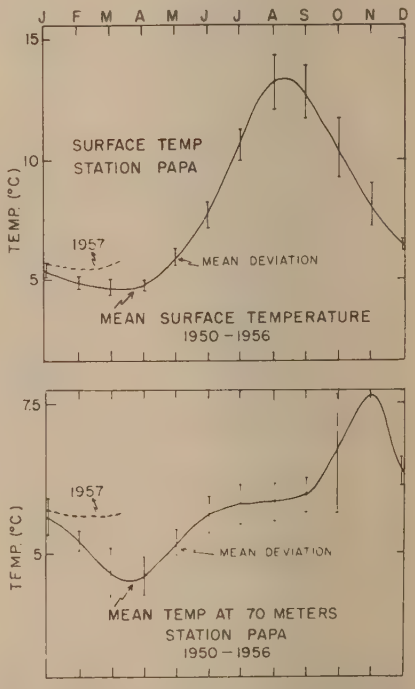

Fig. 7. Monthly mean temperatures, 1950 to 1956, (solid line) and temperatures in early 1957 (broken line) at weather station "PAPA" (50°N., 145°W.)

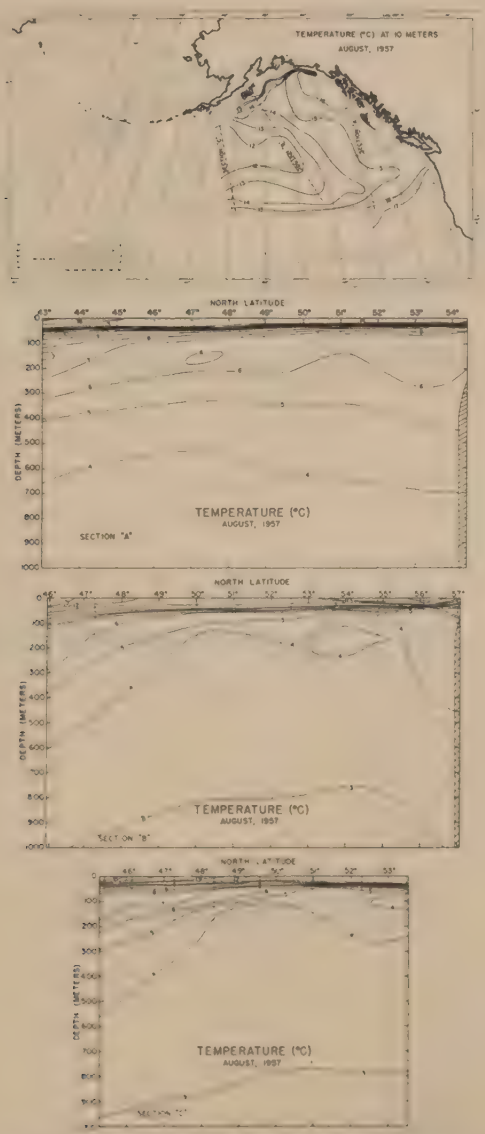

Fig. 8. Temperatures in the eastern North Pacific in August, 1957.

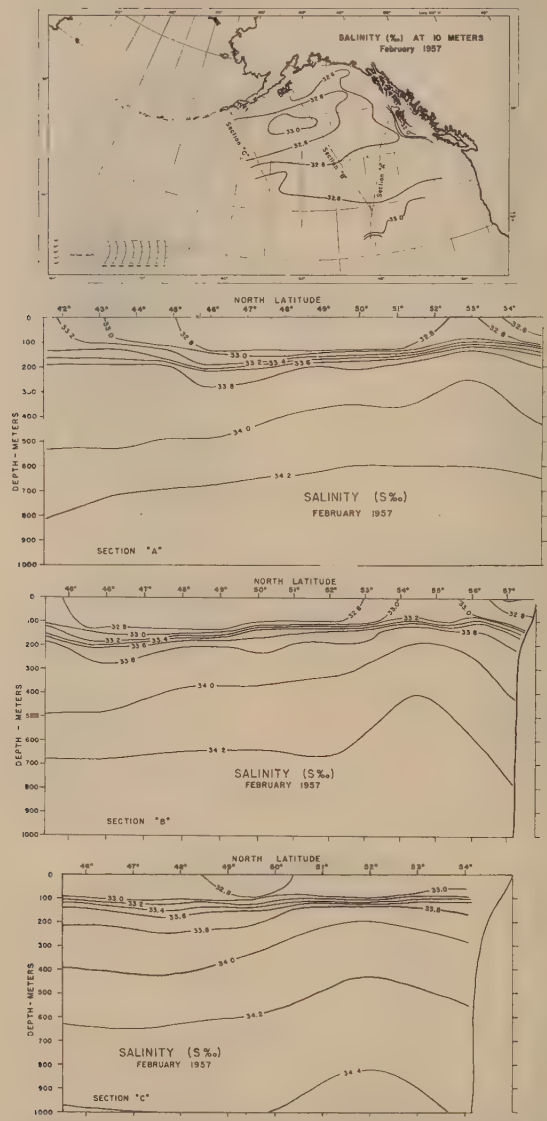

Fig. 9. Salinities in the eastern North Pacific in February, 1957.

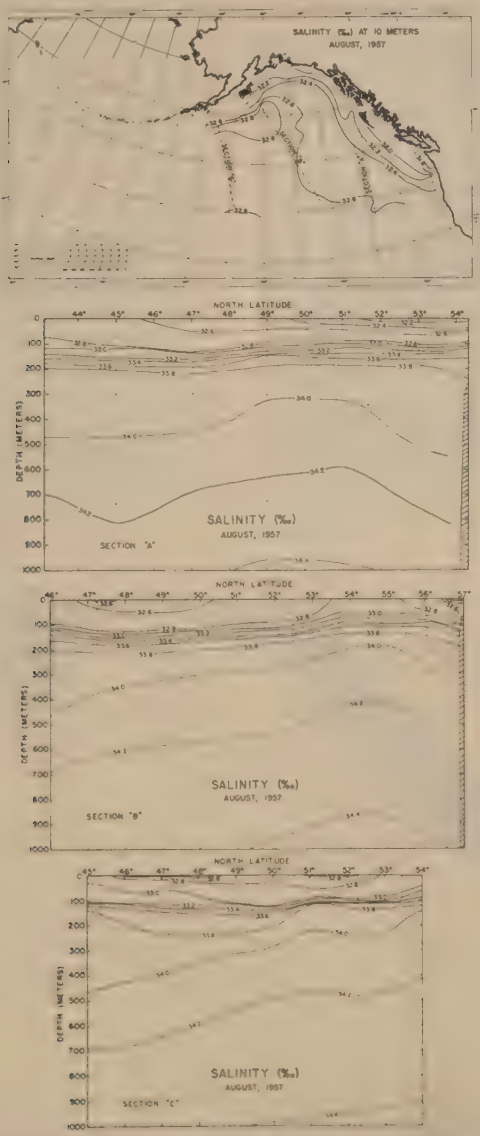

Fig. 16. Salinities in the eastern North Pacific in August, 1957.

Currents. The dynamic computations on the August, 1957, cruise have not yet been completed. They will be shown in a later report. The computations have, however, been made for the winter data (February, 1957) and are shown in Figure 11. It shows that the northern side of the West Wind Drift Current enters the area from the southwest. Most of this water circulates anti-clockwise around the cold centre in the Gulf of Alaska. The current becomes narrower and more intense in the north and along the Alaskan Peninsula. Water also enters along the southern limit of the area, moves towards the American coast and then turns southward. This is evidently the edge of the Kuroshio extension which just intrudes the area before turning south to join the California Current. The approaches to the Canadian coast constitute the region of divergence between the northern and southern (sub-Arctic and sub-Tropic) limits of the West Wind Drift.

Chemical properties and plankton. The concentration of dissolved oxygen is between 80% and 100% of saturation throughout the upper zone. It is highest in the cold centre and lowest near the coast and along the southern side of the region. It decreases markedly in the halocline, and continues to decrease with depth in the deep zone. There is a dome of very low oxygen concentration coincident with the dome of deep cold water.

The concentrations of inorganic phosphate and silicitate are high in the upper zone waters, increasing from the limits of the area toward the cold centre. In the deep zone there is a dome of extraordinarily high concentration of these constituents, coincident with the dome of deep cold water.

The concentrations of dissolved nitrite are low and are frequently zero in both upper and deep zones. There is, however, a thin layer of very high concentration coincident with the lower part of the thermocline over the whole area.

In the upper 150 meters of depth there was about 20 times as much plankton in summer as in winter. This seasonal difference decreased with depth to the limit of sampling at 1,000 meters. There was more plankton in the coastal approaches than in mid-ocean.

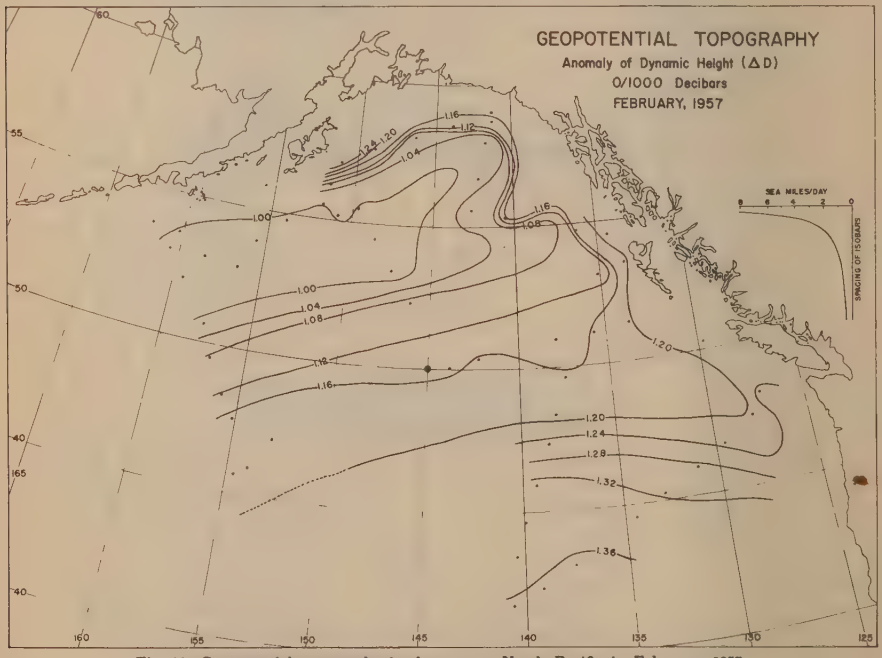

Fig. 11. Geopotential topography in the eastern North Pacific in February, 1957.

III. THE RESEARCH PROGRAM OF THE NORTH PACIFIC FISHERIES COMMISSION

A. PROGRESS IN 1958 IN CANADIAN RESEARCH ON PROBLEMS RAISED BY THE PROTOCOL

As part of the Commission's program of research on problems raised by the Protocol, Canada continued the following lines of investigation in 1958: (1) high-seas fishing to study salmon distribution and obtain samples for study, (2) studies of the parasites of sockeye and pink salmon and of chum salmon scales, to distinquish stocks of various river origins and discover their distribution on the high seas, and (3) study of the oceanography of the North Pacific to provide background for understanding salmon distribution and movements and their variations. Canada also co-operated in other phases of the program by providing material for study by United States and Japan and by efforts to recover tags placed on salmon on the high seas. Studies of the skeletal anatomy of salmon are being discontinued but preparation of reports is extending into 1959.

1. HIGH-SEAS FISHING TO STUDY DISTRIBUTION AND OBTAIN SAMPLES

Canada's high-seas fishing operations in 1958 were carried out by the *Key West II* and the *Fort Ross*. The ships left Nanaimo on May 7 and returned from their final cruises on August 28 and 25 respectively.

The fishing gear consisted mainly of surface gillnets, of mesh size 5¼", 4½", 3¼" and 2½", as used in 1957. A maximum of thirty-six 50-fathom shackles of this gear was set at one time. Additional surface gear carried in 1958 included 9" and 6¼" gillnets, each 200 fathoms long. Each vessel also carried a net capable of being fished at various depths below the surface. This net was 40 feet in depth and consisted of 100 fathoms of 2½" mesh, 100 fathoms of 3¼" mesh and 200 fathoms of 4½" mesh.

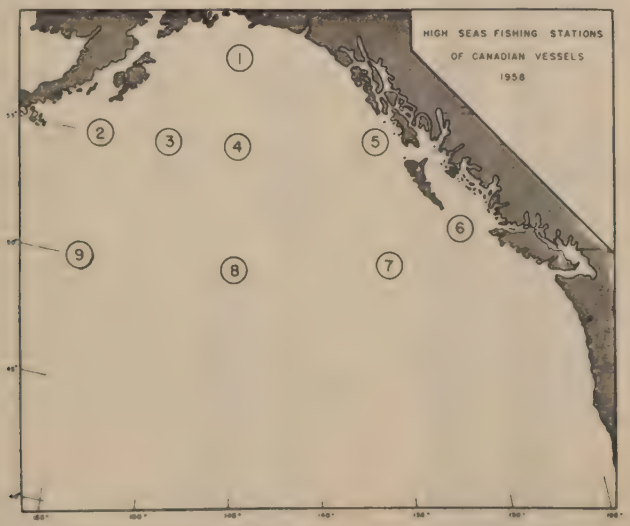

FIGURE 1. High seas fishing stations of Canadian vessels, 1958

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FRB No. 657

Plankton and oceanographic observations, the latter limited to temperature and salinity, were also made in association with the high-seas fishing. Plankton nets were similar to those used in 1957. In 1958, however, mid-water trawls of Isaacs-Kidd pattern were also used.

Fishing was done at 9 stations within a region bounded on the south by latitude 50° N., on the west by longitude 155°W. and on the north and east by the North American coastline (Fig. 1). All these stations were occupied during the earlier part of the season (May-June) and all except Station 3 were again occupied briefly between the end of July and the end of August. In addition to these high-seas stations, a few sets were made at the end of July by the *Key West II* in coastal waters near the west coast of the Queen Charlotte Islands.

Much unfavourable weather was encountered during the fishing season. Because of this and related time losses occasioned by loss or damage of gear, the actual time spent in fishing was less than in 1957. Only 38 surface sets (excluding coastal operations) were made by the two vessels, as against 70 in the previous year. The number of successful sets was reduced especially during the late summer coverage of the high-seas stations.

Salmon Catches and Distribution. Catches and associated data for each set are shown in Table 1. The composition of the total catch by species is shown in Table 2, where comparative information for 1957 and 1956 is given. A main feature of the 1958 catch was the greatly increased percentage of sockeyes, with a corresponding reduction in the relative abundance of all the other species, except chinook which appeared in very small numbers each year.

In Table 3 the early summer catches for each station are summarized, after weighting for amount of gear used and length of time for which nets were set. Sockeye abundance increased with distance north and west from the British Columbia coast.

TABLE 1. Salmon catches by Canadian research vessels in 1958.

	Date	Position		No. of shackles	Surf. temp. (°C)	Depth interval (ft.)	Salmon					
		Lat.(°N)	Long.(°N)				Sockeye	Pink	Chum	Coho	Chinook	Total
Key West II												
Surface nets	May 11	51°00′	130°00′	44	10.1		3	2	1	1	..	7
" "	17	50°00′	135°00′	40	8.7		1	2	..	9	..	12
" "	18	50°00′	135°00′	40	9.4		5	10	64	21	..	100
" "	19	50°00′	135°00′	40	9.5		1	1	8	4	..	14
" "	28	51°00′	130°00′	40	11.1		3	7	5	6	..	21
" "	June 1	55°00′	145°00′	44	8.5		33	4	15	1	..	53
" "	2	55°00′	145°00′	38	8.7		25	5	4	..	..	34
" "	4	58°00′	145°00′	32	10.1		113	7	26	..	..	146
" "	5	58°00′	145°00′	32	10.2		137	31	19	2	..	189
" "	13	55°00′	135°00′	32	11.0		..	..	..	3	..	3
" "	14	55°00′	135°00′	36	11.1		1	..	..	..	..	1
" "	15	55°00′	135°00′	36	11.6		31	81	23	23	..	158
" "	16	55°00′	135°00′	36	11.7		3	6	5	9	..	23
" "	Aug. 8	51°00′	130°00′	4*	14.9		21	..	2	..	..	23
" "	9	50°00′	135°00′	4*	15.5		..	..	..	..	..	..
" "	15	55°00′	135°00′	4*	14.8		..	..	1	..	..	1
" "	16	55°00′	135°00′	12	14.3		1	..	..	..	..	1
" "	23	58°00′	145°00′†	12	14.7		..	..	..	..	..	..
" "	24	58°00′	145°00′†	4*	13.9		..	..	6	1	..	7
Sunken nets	May 17	50°00′	135°00′		8.7	60-100	..	..	..	1	..	1
" "	18	50°00′	135°00′		9.4	140-180	1	..	1	..	..	2
" "	19	50°00′	135°00′		9.5	60-100	..	..	..	..	..	..
" "	28	51°00′	130°00′		11.1	20-60	..	..	..	..	..	..
" "	June 1	55°00′	145°00′		8.5	20-60	2	..	2	..	..	4
" "	2	55°00′	145°00′		8.7	60-100	..	..	..	..	..	..
" "	4	58°00′	145°00′		10.1	20-60	21	1	4	..	..	26
" "	5	58°00′	145°00′		10.2	60-100	9	..	..	..	..	9
" "	14	55°00′	135°00′		11.1	20-60	..	..	..	..	..	..
" "	16	55°00′	135°00′		11.7	60-100	..	..	..	..	..	..
" "	Aug. 7	51°00′	130°00′		15.2	0-40	..	..	..	..	..	..
" "	8	51°00′	130°00′		14.9	20-60	15	..	..	..	..	15
" "	9	50°00′	135°00′		15.5	20-60	..	..	..	..	..	..
" "	15	55°00′	135°00′		14.8	20-60	2	..	..	..	..	2
" "	16	55°00′	135°00′		14.3	0-40	12	..	2	1	..	15
" "	23	58°00′	145°00′†		14.7	0-40	..	..	..	..	..	..
" "	24	58°00′	145°00′†		13.9	20-60	..	..	4	..	..	4

Continued . . .

TABLE 1. (continued) Salmon catches by Canadian research vessels in 1958.

	Date	Position		No. of shackles	Surf. temp. (°C)	Depth interval (ft.)	Salmon					
		Lat.(°N)	Long.(°N)				Sockeye	Pink	Chum	Coho	Chinook	Total
Fort Ross												
Surface nets	May 17	50°00′	135°00′	36	9.6		3	3	1	5	..	12
" "	19	50°00′	135°00′	36	9.4		..	..	..	..	..	..
" "	24	50°00′	145°00′	36	7.6		41	4	15	2	1	63
" "	25	50°00′	145°00′	36	7.7		41	4	26	4	..	75
" "	27	50°00′	145°00′	35	7.9		78	11	45	..	..	134
" "	June 7	55°00′	150°00′	35	8.1		101	..	26	..	..	127
" "	8	55°00′	150°00′	35	7.8		37	1	8	..	..	46
" "	9	55°00′	150°00′	34	8.2		34	1	25	..	..	60
" "	10	55°00′	150°00′	32	8.3		155	5	12	..	..	172
" "	15	55°00′	155°00′	34	7.8		146	9	13	..	..	168
" "	16	55°00′	155°00′	18	7.4		35	1	3	..	..	39
" "	23	50°00′	155°00′	22	7.6		25	9	13	..	..	47
" "	24	50°00′	155°00′	22	7.8		61	3	41	..	3	108
" "	25	50°00′	155°00′	22	8.1		36	11	14	..	1	62
" "	26	50°00′	155°00′	1	8.5		1	..	..	..	..	1
" "	July 26	50°00′	145°00′	17	13.7		1	..	..	..	..	1
" "	Aug. 1	50°00′	155°00′	18	10.2		1	..	47	..	..	48
" "	15	55°00′	155°00′	14	11.0		3	1	25	2	..	31
" "	18	55°00′	145°00′	14	13.4		..	..	1	..	..	1
Sunken nets	May 17	50°00′	135°00′		9.6	20-60	..	..	1	..	..	1
" "	18	50°00′	135°00′		9.5	100-140	1	..	..	1	..	2
" "	24	50°00′	145°00′		7.6	20-60	4	..	2	..	..	6
" "	27	50°00′	145°00′		7.9	60-100	..	..	..	..	..	..
" "	June 7	55°00′	150°00′		8.1	60-100	3	..	1	..	..	4
" "	19	50°00′	155°00′		7.4	20-60	1	5	..	..	..	6
" "	23	50°00′	155°00′		7.6	20-60	1	1	..	..	..	2
" "	24	50°00′	155°00′		7.8	60-100	..	1	..	..	..	1
" "	26	50°00′	145°00′		13.7	0-40	..	..	..	..	..	..
" "	Aug. 1	50°00′	155°00′		10.2	0-40	..	..	1	..	..	1
" "	15	55°00′	155°00′		11.0	0-40	2	1	8	..	..	11
" "	18	55°00′	145°00′		13.4	20-60	..	..	1	..	..	1
Totals—*Fort Ross*							811	71	329	14	5	1230
Key West II							440	157	192	82	..	871
Grant Total							1251	228	521	96	5	2101
%							59.5	10.9	24.8	4.6	0.2	100.0

* 6¼" net. No standard surface nets used.
† Approximate position.

TABLE 2. Salmon catches in the Northeast Pacific.

Year	Sockeye		Pink		Chum		Coho		Chinook		Total
	No.	%	No.	%	No.	%	No.	%	No.	%	
1956	200	21.2	250	26.5	349	37.0	139	14.7	5	0.5	943
1957	997	27.2	519	14.1	1798	48.9	357	9.7	5	0.1	3676
1958	1251	59.5	228	10.9	521	24.8	96	4.6	5	0.2	2101

TABLE 3. Surface catch per 900 fathoms of gillnet per 10-hours night set, with equal representation of 2½", 3¼", 4½" and 5¼" mesh.

Ship	Station	No. of sets	Position		Weighted catch (numbers of fish)					
			Lat. N.	Long. °W.	Sockeye	Pink	Chum	Coho	Chinook	Total
KW	1	2	58	145	51	8	10	1	..	70
FR	2	2	55	155	62	2	4	..	..	68
FR	3	4	55	150	41	1	8	..	..	50
KW	4	2	55	145	10	2	4	1	..	17
KW	5	4	55	135	7	13	4	6	..	30
KW	6	2	55	130	2	3	2	2	..	9
KW, FR	7	4	50	135	1	2	7	3	..	13
FR	8	3	50	145	21	4	11	1	..	37
FR	9	3	50	155	34	4	14	..	1	53

Pink salmon were most plentiful at the northern and northeastern stations. Chum salmon (when all age groups are considered) were spread fairly evenly over the region sampled. Cohoes were commoner at the eastern stations, i.e. towards the coasts of British Columbia and southeast Alaska. Late-season sampling was too scanty to provide reliable information on distribution and abundance.

The sets made with the sunken nets are listed in Table 1. The catches are not considered to provide any reliable information on the relative abundance of salmon at different depths. Various difficulties in the use of the sunken nets were encountered. Unfavourable weather conditions curtailed the program. It proved difficult or impossible to fish the sunken net effectively in conjunction with the large amount of surface gear which was considered necessary to provide adequate samples. Certain features of the construction of the nets also militated against their successful operation. Further investigation of the vertical distribution of salmon is planned for 1959.

Age Composition. The age composition of sockeye and chums caught in the 1958 high-seas fishing operations is given in Tables 4 and 5.

Among sockeyes, 4_2 fish formed the largest age group at all stations except No. 1. The predominance of this group in high-seas catches preceded an extremely heavy run of fish of this category to the Fraser River, B.C. and it is thought that some of the sockeyes caught on the high seas as far west as longitude 155°W. were Fraser River fish. However, the increased high-seas sockeye catches of 1958 (as compared with 1957) were largely due to an increase in the number of 5th and 6th year fish. These presumably originated in northern British Columbia and Alaska.

Chum catches showed a preponderance of 3rd-year fish at the southern and western stations. In the earlier operations 4th-year fish were in a majority towards the northern and eastern shorelines. Fifth-year fish were not represented in the later catches.

Plankton and Feeding of Salmon. Plankton collections made by Canadian research vessels in 1958 included 260 vertical tows from 150 meters to the surface. These permit some comparison of the standing crop of zooplankton by region and time.

The seasonal pattern of the crop in the Gulf of Alaska shows a winter minimum extending from December until early March. After a very rapid increase in numbers in March and April, a maximum is attained in May or early June. August marks the summer minimum but August is the only month in which observations are sufficiently

TABLE 4. Age composition of sockeye catches in the Northeast Pacific, 1958.

Period	Station	Age												Unknown	Total
		2_1	3_1	3_2	4_1	4_2	4_3	5_2	5_3	6_2	6_3	6_4	7_3	7_4	
Early	1	..	..	1	..	54	3	126	38	..	33	12	1	1	11	280
	2	..	..	2	1	93	3	32	22	..	9	2	..	..	17	181
	3	..	..	1	..	160	2	79	38	..	28	2	..	1	19	330
	4	..	..	..	..	28	..	6	11	1	3	4	..	..	7	60
	5	5	..	2	..	9	..	6	3	..	..	..	..	..	10	35
	6	..	..	..	..	1	1	1	1	..	..	..	..	..	2	6
	7	..	..	..	..	7	..	..	4	..	..	..	..	..	1	12
	8	..	3	3	..	109	1	3	27	..	1	3	..	..	14	164
	9	..	..	34	1	61	12	2	6	1	..	..	..	..	8	125
	Totals	5	3	43	2	522	22	255	150	2	74	23	1	2	89	1193
	%	0.4	0.3	3.6	0.2	43.9	1.8	21.4	12.6	0.2	6.2	1.6	0.1	0.2	7.5	100.0
Late	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	2	..	..	..	..	2	2	..	1	..	..	..	..	..	..	5
	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	5	..	..	..	..	15	..	..	..	..	..	..	..	..	..	15
	6	..	..	..	..	30	..	2	4	..	..	..	..	..	..	36
	7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	8	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1
	9	..	..	1	..	..	..	..	..	..	..	..	..	..	..	1
	Totals	..	..	1	..	48	2	2	5	..	..	..	..	..	..	58
	%	..	..	1.7	..	82.8	3.4	3.4	8.6	..	..	..	..	..	..	99.9

TABLE 5. Age composition of chum salmon catches in the Northeast Pacific, 1958.

Period	Station	Age					
		2	3	4	5	Unknown	Total
Early	1	..	8	32	5	4	49
	2	..	11	1	2	2	16
	3	..	38	23	5	6	72
	4	..	7	12	..	2	21
	5	13	1	9	..	5	28
	6	1	1	4	..	..	6
	7	3	61	3	..	8	75
	8	1	49	30	4	4	88
	9	..	44	19	1	5	69
	Totals	18	220	133	17	36	424
	%	4.3	52.0	31.4	4.0	8.3	100.0
Late	1	..	3	6	..	1	10
	2	7	22	3	..	1	33
	3	..	..	..	..	..	..
	4	..	1	1	..	..	2
	5	..	2	1	..	..	3
	6	..	..	2	..	..	2
	7	..	.	..	.	.	.
	8	..	..	..	..	..	..
	9	9	33	2	..	4	48
	Totals	16	61	15	..	6	98
	%	16.3	62.3	15.3	..	6.1	100.0

numerous and consistent to permit a year-to-year comparison over most of the Gulf of Alaska. Figure 2 indicates the relative size of the standing crop during this month in the years 1956, 1957 and 1958. It will be seen that 1956 and 1958 are essentially similar, while in 1957 the standing crop was approximately half as great. This reduction may have been due to greater cloud cover, which would tend to prevent full utilization of nutrients by the phytoplankton and thus limit zooplankton production.

Examination of sockeye and pink salmon stomachs indicates that these species in the Gulf of Alaska relied more on copepods and less on euphausians in 1957 than in 1956 or 1958, suggesting that the latter were at even a lower level than the smaller zooplankton.

Salmon Distribution and Water Temperatures. Studies of salmon and water temperature have shown the need for considering maturing and immature fish separately. In the Gulf of Alaska relatively good catches of maturing fish have been made in waters up to 15°C. Catches of immature sockeye and chum salmon, particularly the latter species, appear to be related to depth of the 5°, 6° and 7°C. isotherms. Where these isotherms are closer to the surface, catches are greater, the correlation being with the 6° isotherm.

Comprehensive Study of Salmon Distribution. Analysis of United States, Japanese and Canadian catch data collectively shows that the distribution of sockeye and chum salmon in the Bering Sea and North Pacific Ocean in 1956 differed to some extent from that in 1957. In general, in the latter year, the areas of abundance of these species in each month from May to September were located farther to the westward. It was also observed from the monthly distribution of surface isotherms for this period, based on data collected by the fishing and oceanographic vessels of each National Section, that the waters were warmer farther westward in 1957 than in 1956.

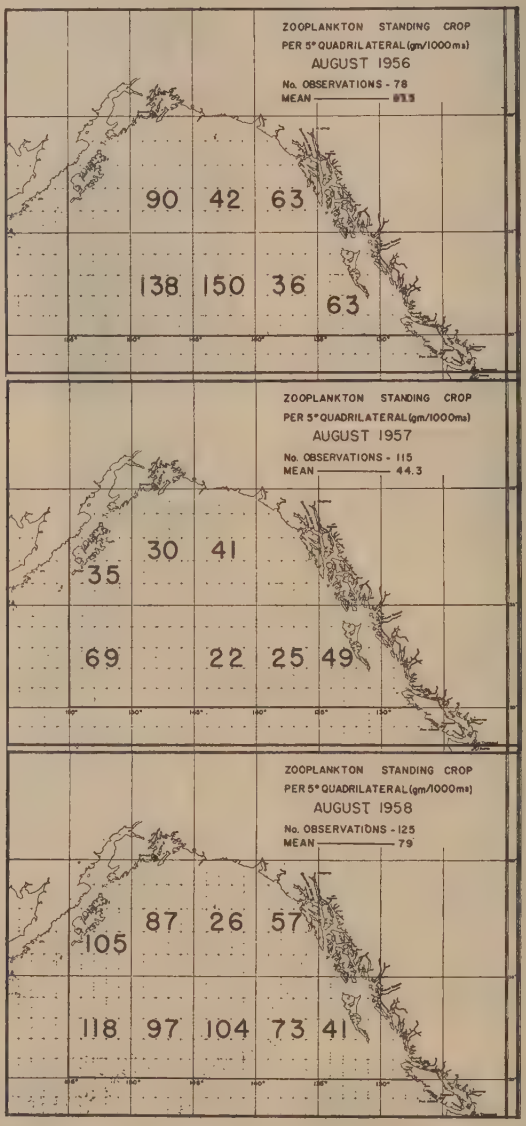


FIGURE 2. Zooplankton standing crop in Gulf of Alaska for the years 1956-1958

2. STUDY OF SALMON PARASITES TO DISTINGUISH STOCKS

A. Sockeye Salmon. Since 1955 investigations of the parasites of sockeye salmon have been underway to assess their value as indicators of the continent of origin of sockeye taken on the high seas.

Sampling of adult sockeye along the North American coast and of sockeye taken on the high seas increased from 1955 through 1957; smolt sampling from North America remained about the same for the 3 years. From 1957 collections, 1,903 adult or sub-adult sockeye were examined in samples from across the North Pacific and Bering Sea, from the Sea of Okhotsk and from the main spawning runs or fisheries of North America. Three hundred and fifty smolts were also examined from collections made in 1957. Details of the geographical location, date of catch and numbers of sockeye examined from each locality are given in Tables 6 and 7. Figure 3 also shows pictorially the distribution of coastal adult and high-seas samples in 1957.

Japan, United States and Canada have cooperated in providing samples of sockeye from the North Pacific and Bering Sea from research vessel operations. In addition, Japan has provided samples from commercial fishing operations in the North Pacific and Bering Sea west of 175° W. longitude, and in the Sea of Okhotsk. Adult sockeye from coastal areas, and smolts have been collected by the United States and Canada from various North American localities.

Of the six species of parasites listed in last year's annual report only four continue to give evidence of the continent of origin of sockeye infested with them. These are *Triaenophorus crassus* (acquired in fresh water) and *Tubulovesicula lindbergi* (acquired in the sea), characteristic of certain North American stocks, and *Dacnitis truttae* (acquired in fresh water) and *Hemiurus levinseni* (acquired in the sea), indicative of Asiatic origin.

TABLE 6. Locality, date of catch and size of coastal adult and high-seas sockeye samples examined for parasites in 1957. (The numbers preceding the locality refer to the numbered positions in Figure 3)

	Locality	Date of catch		Number of sockeyes		Locality	Date of catch		Number of sockeyes
1	50°52'N., 153°28'E.	4-VII	1957	25	31	53°N., 175°W.	9-VI	1957	25
2	51°16'N., 153°39'E.	14-VII	"	25	32	53°N., 175°W.	10-VI	"	24
3	52°08'N., 154°15'E.	24-VI	"	25	33	56°N., 175°W.	15-VI	"	40
4	49°14'N., 158°11'E.	10-VII	"	23	34	56°N., 175°W.	30-VI	"	21
5	51°13'N., 159°45'E.	24-VII	"	25	35	56°N., 173°W.	17-VI	"	25
6	51°33'N., 161°40'E.	20-VII	"	25	36	56°N., 170°W.	18-VI	"	25
7	51°39'N., 162°16'E.	1-VII	"	25	37	55°N., 168°W.	22-VI	"	30
8	52°33'N., 166°04'E.	20-VI	"	25	38	56°N., 168°W.	21-VI	"	16
9	48°08'N., 167°40'E	21-V	"	25	39	55°N., 166°W.	24-VI	"	14
10	51°40'N., 167°27'E.	28-VI	"	25	40	56°N., 166°W.	23-VI	"	19
11	52°31'N., 168°27'E.	25-V	"	25	41	50°N., 165°W.	9, 20, 21-VIII	"	24
12	58°12'N., 166°48'E.	15-VII	"	26	42	53°N., 165°W.	31-VII	"	15
13	57°02'N., 169°11'E.	12-VII	"	19	43	53°N., 165°W.	24-VII	"	25
14	54°51'N., 170°24'E.	6-VII	"	20	46	54°N., 160°W.	15-VII	"	27
16	50°N., 175°E.	12-VI	"	25	47	52°N., 160°W.	17-VII	"	25
17	50°N., 175°E.	10, 11, 12-VI	"	43	54	50°N., 155°W.	23-VI	"	22
18	50°N., 175°E.	9-VIII	"	25	55	50°N., 155°W.	30, 31-VII	"	25
19	50°N., 175°E.	20-VIII	"	25	56	55°N., 155°W.	30-VI	"	23
20	51°30'N., 175°E.	19-VIII	"	25	57	55°N., 155°W.	18, 19-VIII	"	25
21	53°N., 175°E.	14-VI	"	25	60	50°N., 145°W.	27, 29-VI	"	14
22	53°N., 175°E.	17-VIII	"	25	61	50°N., 145°W.	13-VI	"	23
23	56°N., 175°E.	14-VIII	"	24	62	55°N., 145°W.	11-VII	"	10
24	53°N., 180°	22-VI	"	25	63	55°N., 145°W.	28-VI	"	13
25	51°N., 176°W.	8-VII	"	25	64	58°N., 145°W.	9-VII	"	24
26	50°59'N., 175°10'W.	7-VIII	"	25	67	50°N., 135°W.	20-VII	"	23
27	50°59'N., 175°01'W.	22-VIII	"	25	68	50°N., 135°W.	2-VII	"	25
28	50°N., 175°W.	9-VIII	"	25	69	50°N., 135°W.	8-VI	"	21
29	50°N., 175°W.	20-VIII	"	25	70	55°N., 135°W.	30, 31-VII	"	20
30	53°N., 175°W.	8-VI	"	24	71	55°N., 135°W.	14-VI	"	25

TABLE 7. Locality, date of catch and size of sockeye smolt samples examined for parasites in 1957.

Locality	Date of catch		Number of sockeyes
Wood River, Bristol Bay, Alaska	11-VII	1957	25
Kvichak River, Bristol Bay, Alaska	31-V	"	25
Naknek River, Bristol Bay, Alaska	13 to 19-VI	"	25
Ugashik River, Bristol Bay, Alaska	22-V	"	25
Chignik Lake, Alaska Peninsula	4-VI	"	25
Karluk River, Kodiak Is., Alaska	26-V	"	25
Helm Bay, southeast Alaska	27-VI	"	25
Babine Lake, Skeena R., B.C.	30-V	"	25
Nilkitkwa Lake, Skeena R., B.C.	31-V	"	25
Port John, central British Columbia	19-V	"	25
Shushwap Lake, Fraser R., B.C.	May	"	25
Chilko River, Fraser R., B.C.	7-V	"	25
Cultus Lake, Fraser R., B.C.	26-V	"	25
Baker R., (Skagit R.) Washington	26-V	"	25
Total number of smolts in 1957			350

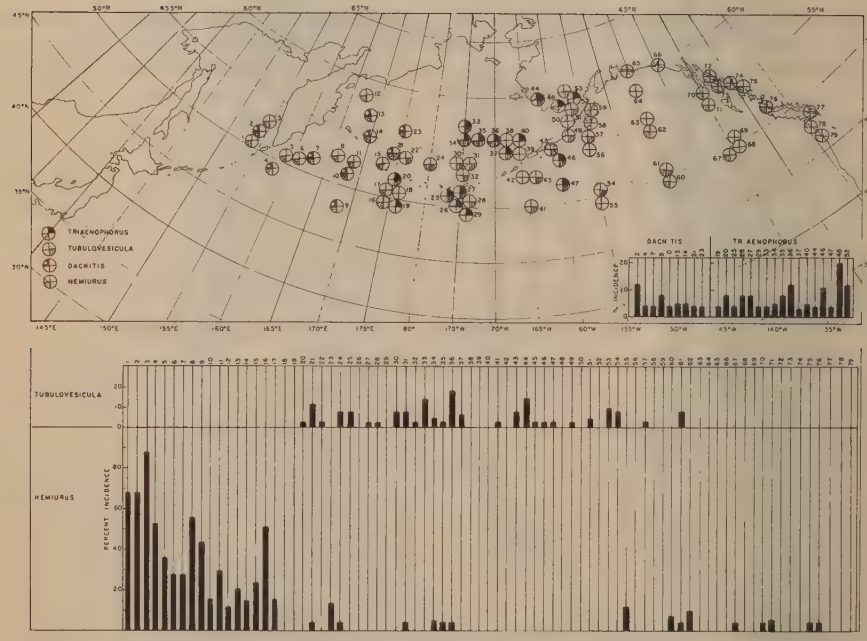


FIGURE 3. Distribution of sockeye salmon samples in 1957 with occurrence and incidence of *Triaenophorus*, *Dacnitis*, *Tubulovesicula* (mature) and *Hemiurus* in these samples.

The occurrence of the latter parasite in some southeast Alaska and British Columbia areas will be discussed later. On the basis of the 1957 samples, larvae of *Anisakis* sp. and *Echinorhynchus gadi* no longer qualified as indicators of the continent of origin of sockeye captured on the high seas.

i. Distribution of Triaenophorus, Dacnitis, Tubulovesicula and Hemiurus in sockeye.

Triaenophorus crassus. This larval cestode, acquired by sockeye in fresh water, has been found in smolts and returning spawners only in certain areas in western Alaska, mainly in Bristol Bay. The specific localities in which *Triaenophorus* has been observed in the years 1955 through 1957 are the Wood River, Naknek River and Ugashik River in Bristol Bay, and the Kuskokwim River, north of Bristol Bay. The incidence is greatest in the Wood River, less in the Naknek River, and least in the Ugashik and Kuskokwim Rivers. In the high-seas samples it was observed from 150° W. longitude in the Gulf of Alaska to 175°E. longitude, south of the Aleutians, and from 175°W. longitude, eastward, in the Bering Sea. *Triaenophorus* was not observed west of 175 E. longitude in any of the three years of investigation.

The distribution and incidence of this parasite in the coastal adult and in the high-seas samples collected in 1957 are shown in Figure 3. Figure 4 illustrates the distribution of all sockeye (including smolts) infected with *Triaenophorus* from the years 1955 through 1957 and gives the incidence of *Triaenophorus* in each sample, the date of collection and the age of the infected sockeye.

Tubulovesicula lindbergi. Mature specimens of this marine trematode were found in some of the North American coastal samples, particularly in central and western Alaska, in all three years. On the high seas adult *Tubulovesicula* were observed in many of the sockeye samples south of the Aleutians, from 171°E. longitude to 150°W. longitude (rarely at 145°W.) in the Gulf of Alaska, and from 175 E. longitude to 165°W. longitude in the Bering Sea. They were absent from all samples examined from 170°E. longitude to the Sea of Okhotsk. (See Fig. 3.)

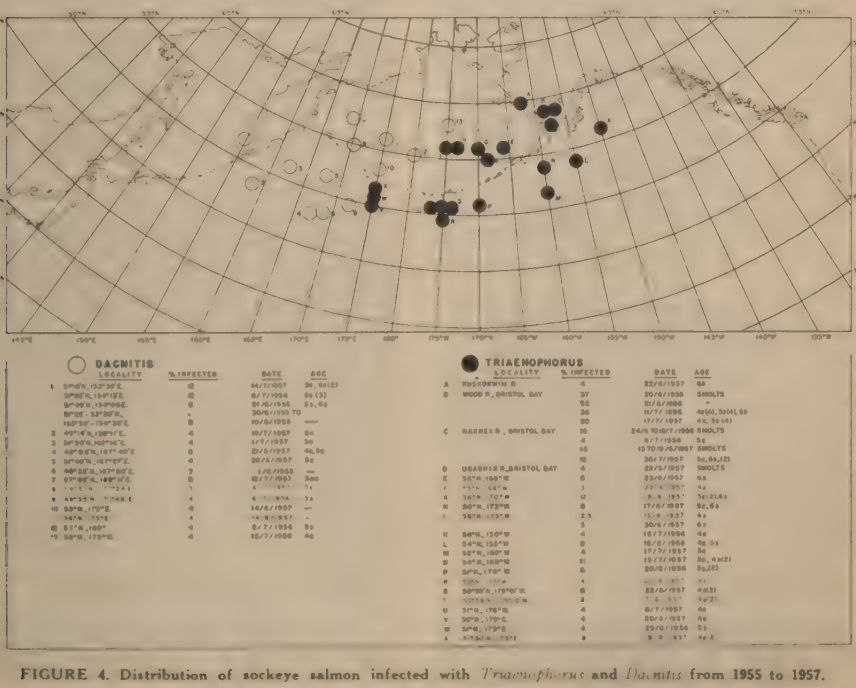


FIGURE 4. Distribution of sockeye salmon infected with *Triaenophorus* and *Dacnitis* from 1955 to 1957.

Small, immature specimens of this species were also found in mature sockeye from some North American coastal areas, particularly in southeast Alaska and British Columbia. It appears that *Tubulovesicula* in these more southerly areas resulted from infection of the mature sockeye after re-entry into coastal waters, and the occurrence of immature *Tubulovesicula* cannot be considered to show relationship of these fish to high-seas sockeye infected with mature *Tubulovesicula.*

Dacnitis truttae. This nematode, with a freshwater life cycle, occurred in sockeye samples from the Okhotsk Sea to 172°E. longitude, south of the Aleutians, and to 175°W. longitude in the Bering Sea. It was not present in any of the high-seas samples east of these longitudes, nor in any of the North American coastal adult and smolt samples. The distribution and incidence of *Dacnitis* in the 1957 samples is given in Figure 3. Figure 4 shows the accumulated distribution of *Dacnitis*-infected sockeye for the three years, with data on incidence, date of catch and age of the fish.

Hemiurus levinseni. This marine trematode was noted in all high-seas samples in the three years, from the Sea of Okhotsk to 170°E. longitude, at latitudes between approximately 48°N. and 58°N. It occurred in some of the samples from 171°E. to 175°E. longitude, south of the Aleutians, and in many of the 1957 samples in the Bering Sea from 175°E. to 170°W. longitude. In the Bering Sea samples east of 175°E. to 170°W. its incidence was considerably less than in more westerly areas. South of the Aleutians and the Alaska Peninsula, *Hemiurus* was absent from all samples from 180° to 160°W. longitude. In the Bering Sea it was absent east of 170°W. longitude.

Hemiurus was found in the Gulf of Alaska, particularly from 150°W., eastward, but in a much lower frequency than in the western Pacific areas. In North American coastal areas it appeared regularly from 1955 through 1957 in some of the samples from southeast of Petersburg, Alaska (ca. 57°N., 133°W.), the incidence in these samples being similar to that in the Gulf of Alaska samples. With two exceptions, *Hemiurus* was absent from all central and western Alaska samples in the three years. In 1955 one sockeye infected with one specimen of *Hemiurus* was found in the Bristol Bay samples, and in 1956 a similarly infected sockeye was found in the sample from the Kuskokwim River, western Alaska.

The distribution and incidence of *Hemiurus* in the 1957 samples is shown in Figure 3.

ii. Are the selected parasites valid indicators of the geographical origin of sockeye taken on the high seas? Two of the four selected parasites are acquired in fresh water before the fish migrate to sea and apparently remain with the fish throughout its life. Because they are restricted in their distribution in sockeye to certain areas of one or other of the two continents they serve as excellent "tags" of the continent of origin of sockeye infected with them.

The other two parasites are acquired in the sea. Although the exact locality in which infection of sockeye by these parasites occurs, the age at which the sockeye become infected and the life span of the parasites in sockeye are unknown, the characteristics of their distribution suggest that they are of value in recognizing the high-seas distribution of stocks from the two continents.

Triaenophorus crassus. This is a larval (plerocercoid) cestode inhabiting the musculature of the sockeye. The life history involves a freshwater fish, *Esox lucius,* in the intestine of which it matures. After the parasite becomes gravid the eggs eventually reach the water where they hatch. The resulting embryos (coracidia) must be ingested by certain species of copepods, mainly *Cyclops,* for continued development. In the copepod they reach the first larval (procercoid) stage. Young sockeye acquire the parasite in fresh water by eating infected copepods, and *Esox* becomes infected by eating fish parasitized with the second larval stage. The important host in this cycle is *Esox.* The parasite will not mature in the intestine of other fishes and hence larval *Triaenophorus crassus* can only occur in sockeye in lakes co-inhabited by *Esox.*

The absence of *Esox* from Pacific drainages (as distinct from Bering Sea drainages) of North America and from the Kamchatka Peninsula, precludes the possibility of the occurrence of this parasite in sockeye from these areas. The absence of *Triaenophorus* from sockeye from North American Pacific drainages has been confirmed by examination of smolts and adults from many localities. Smolts or adults from inshore waters were not available from Kamchatka but the samples from the Sea of Okhotsk and from the far-western Pacific and Bering Sea were all free of *Triaenophorus.* Also, in recent studies of parasites of sockeye from Kamchatka by scientists in the U.S.S.R., *Triaenophorus* is not noted. On the other hand, observations from 1955 through 1957 have indicated that smolts from, and adults returning to, certain western Alaska areas (mainly Bristol Bay) are infected with *Triaenophorus.* Since the parasite cannot be acquired in the sea, the *Triaenophorus* in sockeye returning to spawn after two or three years in the ocean are the same that were acquired in fresh water.

It is concluded that sockeye caught on the high seas, which are infected with *Triaenophorus,* must be of western Alaskan origin, apparently mainly from Bristol Bay.

Dacnitis truttae. This nematode has a freshwater life cycle and lives in the intestine of salmonoid fishes. The intermediate host or hosts, in which larval development takes place, and which transmit the parasite to sockeye, are apparently unknown, but it is presumed that only one intermediate host is required, which is probably an aquatic arthropod.

The parasite was found in the Sea of Okhotsk sockeye samples in each of the years 1955 through 1957 and in many of the sampled localities in the western Pacific and Bering Sea. Recent publications from the U.S.S.R. record *Dacnitis* as a parasite of the sockeye from Kamchatka. The occurrence of this freshwater parasite in sockeye of age 5_2 or 6_2, taken on the high seas, indicates an ability to live in sockeye for at least 3 to 4 years. *Dacnitis* was not found in any of the smolts from, or adults returning to, North American localities. It appears then, that sockeye taken on the high seas parasitized by *Dacnitis* have acquired the parasite in Kamchatka.

Tubulovesicula lindbergi. This is a marine trematode which occurs as an adult in the stomach of sockeye and other fishes. Its life history is unknown, but like practically all other trematodes the first intermediate host is almost certainly a mollusc and probably a gastropod. A second intermediate host which carries the stage infective to sockeye, is probably involved and is probably a crustacean.

Since the life cycle and hosts, the exact locality where infection takes place, and the life span of the adult trematode are unknown, interpretation must be based on the observed distribution of the infected sockeye. As noted in the previous section, mature *Tubulovesicula* occurred in certain stocks returning to spawn in North America and in many of the high-seas samples from North American waters to 171°E. longitude. The parasite was not evident in any of the samples from 170°E. longitude to the Sea of Okhotsk. Furthermore, in high-seas samples in which *Tubulovesicula* occurred in the *Hemiurus* and/or *Dacnitis*, it never occurred in the same individual fish as either of these two parasites, which are considered indicative of Asiatic origin of sockeye. It was, however, recovered from some sockeye simultaneously infected with *Triaenophorus*. Also, the distribution of sockeye infected with *Tubulovesicula* is quite similar to that of sockeye infected with *Triaenophorus*. It thus appears that *Tubulovesicula* is associated with sockeye of North American origin.

Hemiurus levinseni. This is another marine trematode which, in the adult stage, lives in the stomach of sockeye and other fishes. Its life cycle is unknown, but is presumably similar to that postulated for *Tubulovesicula*. The intermediate hosts, particularly the molluscan host, of *Hemiurus* are, however, probably different than those of *Tubulovesicula*. As with *Tubulovesicula*, interpretations must be based on the geographical distribution of sockeye infected with this trematode. It appears to have two main centers of distribution. One is in western waters (presumably Asiatic) and the other is in southern North American localities, the parasite occurring more frequently and abundantly in sockeye from the former area.

In all high-seas areas, other than in the Gulf of Alaska, *Hemiurus* is believed to indicate Asiatic origin of the infected sockeye for the following reasons: the continuous distribution of the parasite in the North Pacific in samples from the Sea of Okhotsk to 175°E. longitude and its absence from 180° to 160°W. longitude; the presence of *Hemiurus* in samples from the Bering Sea from 165°E. to 170°W. longitude and its absence east of 170°W.; the irregular occurrence of *Hemiurus* in samples from the Bering Sea taken between 175°E. and 170°W. longitude and the lower incidence in samples from this area than in samples from west of 175°E. longitude; the almost complete absence of *Hemiurus* from North American coastal samples taken north and west of Petersburg, southeast Alaska (ca. 57°N., 133°W.); and the fact that *Hemiurus* never occurred in the same individual sockeye as *Tubulovesicula* or *Triaenophorus* (although it was found in the same sample) but did occur together with *Dacnitis* in the same fish.

The presence of *Hemiurus* in some sockeye caught in the Gulf of Alaska probably indicates a relationship of these to those from southern North American localities. If it reflected a relationship with sockeye of western Pacific areas we should expect to find sockeye between 180° and 160°W. longitude infected with this parasite which, in fact, has not been observed.

The rare occurrence of *Hemiurus* in the Kuskokwim River, western Alaska, in the 1956 samples, and in Bristol Bay, Alaska, in the 1955 samples, probably does not materially affect the general conclusions regarding the origin of *Hemiurus*-infected sockeye taken on the high seas, but it would seem to leave open the possibility that *Hemiurus* infected sockeye in the Bering Sea could be of western Alaskan origin. However, in 1957, when *Hemiurus* was found in the Bering Sea samples as far east as 170°W. longitude, sockeye samples from western Alaska were entirely free of *Hemiurus*. If any appreciable numbers of *Hemiurus*-infected sockeye from the Bering Sea in 1957 were of Alaskan origin it seems that *Hemiurus* should have appeared somewhere in the 1957 samples from western Alaska. Whether the rare occurrence of *Hemiurus* in western Alaskan sockeye is due to an indigenous low rate of infection or whether it is the result of migration of such sockeye into western waters,

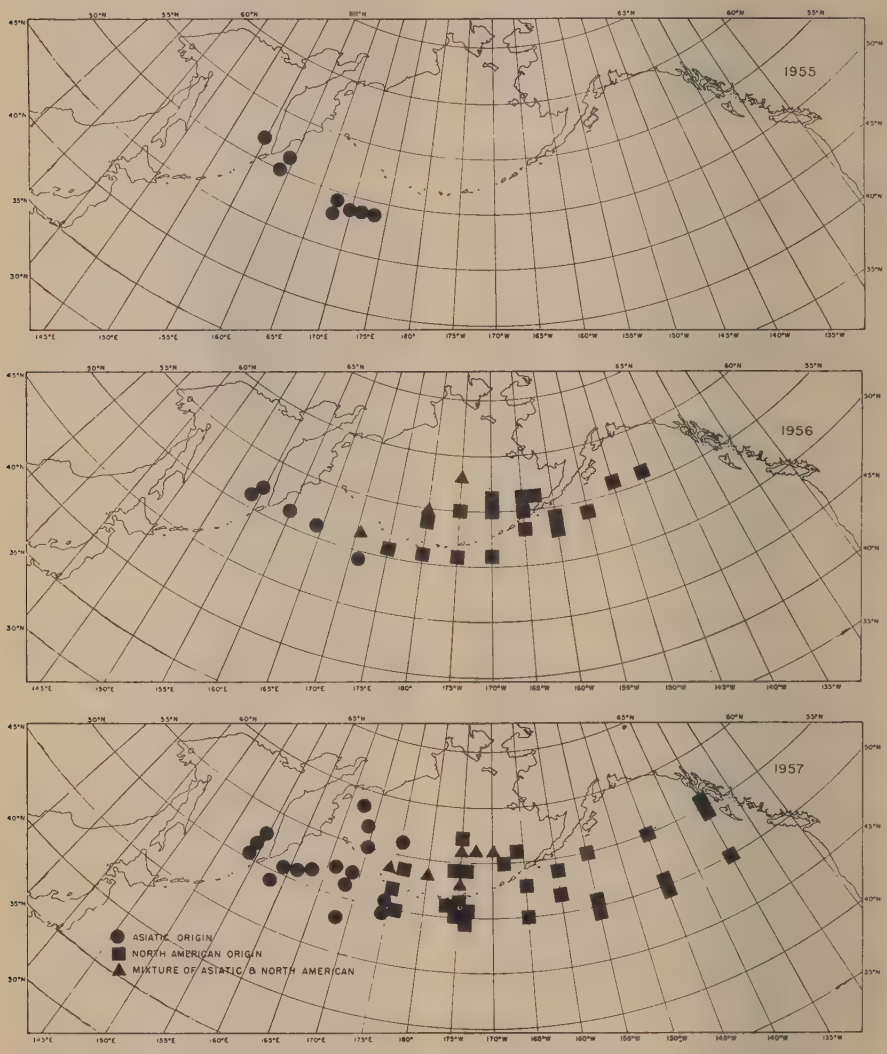


FIGURE 5. The distribution of sockeye salmon of Asiatic and North American origin, as determined by the presence of the parasites *Triaenophorus, Tubulovesicula, Dacnitis* and *Hemiurus,* in high-seas samples collected from 1955 through 1957. (Note: Since in every sample only a portion of the fish possessed parasite characteristics enabling the identification of their continent of origin, these charts refer only to this portion of the samples.)

where *Hemiurus* is apparently readily available to sockeye, is not known.

iii. High-seas distribution of sockeye from North America and Asia as determined from the "indicator" species of parasites. Figure 5 shows the distribution of sockeye salmon of Asiatic and North American origin, as determined by the presence of *Triaenophorus, Tubulovesicula, Dacnitis* and *Hemiurus,* in high-seas samples collected from 1955 through 1957. On the basis of the distribution of these four parasites, the most western extent of North American sockeye was about 171°E. (in 1956) and the most eastern extent of Asiatic sockeye was at 170°W. (in 1957). Between 171°E. and 170°W. some samples showed only the presence of sockeye with North American parasite characteristics, others showed only sockeye with Asiatic characteristics, and some showed the presence of both. Because in all samples a greater or lesser number of individual sockeye do not possess any of the parasite characteristics which enable assignment of the fish to North American or Asiatic origin, it is possible that the range of distribution of stocks from the two continents is even greater than indicated.

The distribution of North American and Asiatic sockeye as suggested solely by the freshwater parasites *Triaenophorus* and *Dacnitis* limits the westward distribution of North American sockeye to 175°E. longitude and the eastward distribution of Asiatic sockeye to 175°W. longitude in the samples studied (see Fig. 4). The extension of this zone of overlap in high-seas distribution of sockeye from the two continents by inclusion of data on the distribution of the marine trematodes *Tubulovesicula* and *Hemiurus* probably has its explanation in the fact that the latter are more abundant and/or more widespread than the freshwater parasites in the stocks from either continent. For example, the incidence of *Hemiurus* is much higher than *Dacnitis* in Asiatic sockeye from the Sea of Okhotsk and off the east coast of Kamchatka. Therefore, when Asiatic stocks intermingle with North American stocks, and hence are "diluted", the probability of encountering *Dacnitis*-infected sockeye in a sample is less than the probability of finding *Hemiurus*-infected sockeye. The possibility that *Dacnitis* and *Hemiurus* do not occur together in all Asiatic stocks could also offer an explanation of the occurrence of only one or the other parasite in a sample of sockeye from the high seas. Again, *Tubulovesicula* is more widely distributed than *Triaenophorus* in samples from North American coastal localities and *Tubulovesicula* may indicate the high-seas distribution of some stocks not simultaneously infected with *Triaenophorus.*

Annual variations. Comparing the three years there appears to be some variation in the extent of distribution of sockeye from the two continents. In 1957 Asiatic sockeye were observed 5° farther east than in 1956 (170°W. compared to 175°W.), and in 1956 North American sockeye were observed 4° farther west than in 1957 (171°E. compared to 175°E.). In 1955 North American sockeye were not observed in the samples at 173°E.

Seasonal variations. In addition to the apparent annual variation, marked seasonal variations were noted at some sampling stations. South of the Aleutians, at 175°E. and 50° to 52°N., in samples collected early in June, 1957, there was no evidence of North American sockeye, but sockeye with Asiatic parasite characteristics were observed. In late August, at the same localities, no Asiatic sockeye were identified, but there was positive evidence of North American sockeye. Whether this same seasonal change in composition of samples occurs to the east or west of 175°E., south of the Aleutians, is unknown, since all samples from east of 175°E. were taken in mid or late summer, and all samples from west of 175°E. were taken in May or June.

Quantitative interpretation. Since only a portion of the fish in any one sample possessed parasite characteristics which enabled assignment to their continent of origin, it is impossible to determine the proportions of North American and Asian sockeye in the total sample from each locality. However, it is probably noteworthy, from a quantitative standpoint, that in the mixing zone, i.e. between 170°W. and 171°E., more sockeye with North American parasite characteristics than with Asiatic parasite characteristics were observed from 170° W. to 180°, but the reverse was true from 175°E. to 171°E.

B. Pink Salmon. The survey of pink salmon parasites has been in operation since 1955. The distribution and abundance of many of the parasites found in pink salmon were not uniform throughout the sampled areas and although differences in parasitism were observed between areas, these differences were not sufficiently stable from year to year to be useful in characterizing pink salmon stocks from the two continents. Studies on the parasites of pink salmon have been discontinued.

3. STUDY OF CHUM SALMON SCALES TO DISTINGUISH STOCKS

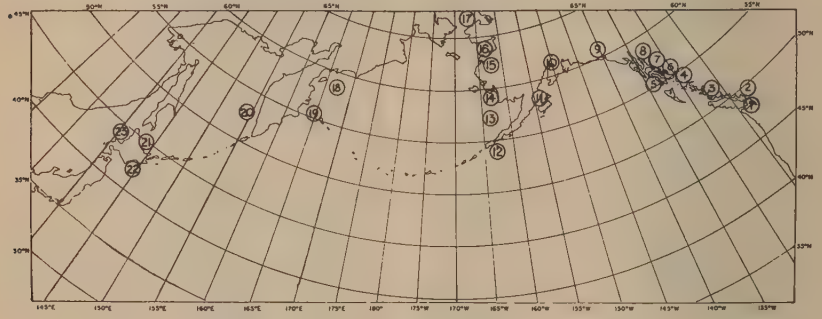
The Canadian study of chum salmon scales to distinguish stocks on the high seas was initiated in 1957. The scales of 3,751 adult chums collected in 1956 from various inshore fishing areas, spawning grounds and high-seas sampling stations were examined.

A. Examination of "Shore" Samples. Samples were obtained from mature chum salmon taken in 23 locations along the North American and Asian coasts. Where possible these samples were

taken in inshore fishing areas or river mouths immediately adjacent to the spawning grounds of the fish. Scale samples from 1,741 adult chum salmon from 6 river systems and 9 inlets on the North American coast and from 3 rivers and 3 high-seas fishing areas close to the Asian coast were examined. The comparative study was restricted to collections made in 1956. The sampling areas and the number of samples obtained from each area are shown in Figure 6.

Preliminary studies of these samples and of a series of samples taken on the Skeena River over a 3-year period (1955-1957) indicated (a) that the scale characters of the first-year zone were less subject to variations associated with the age, sex, and year of origin than were the patterns of the zones of the succeeding years; and (b) that the scale characters of the first-year zone showed a cline with respect to geographic location, whereas the patterns of the succeeding years showed a much less definite systematic trend. For these reasons the study was restricted to variations in first-year scale patterns.

Scales from chums of all ages and both sexes sampled in 1956 in different areas showed marked differences in the numbers of first-year circuli and in the spacing of these circuli. In general, the British Columbia chum salmon were characterized by having many closely packed circuli throughout the entire first-year band. Southeast Alaska fish also had densely packed circuli but the circuli were sparser in the first half of the band than in the second half. Central and western Alaska (including Bristol Bay) fish tended to have less densely packed circuli generally but these were more closely packed in the first half of the first-year band. Fish from the North Bering Sea, Arctic Ocean, and Northwestern Pacific Ocean (i.e. off the coast of Kamchatka and in the Sea of Okhotsk) tended to have sparse, well-separated circuli with no tendency for bunching either in the first or second half of the band. Scales of chum salmon from Japanese rivers were similar to those from Bristol Bay fish, but did not have circuli as closely compacted at the beginning of the first-year band. To illustrate differences in numbers and relative spacing of circuli, the numbers of circuli in the first and last halves of the first-year band and the total number of first-year circuli are illustrated in Figure 7. These figures were derived by pooling all samples within each of the 6 large areas.



Map No.	Location	Number of Samples	Map No.	Location	Number of Samples
1	Puget Sound	68	14	Kuskokwim River	17
2	Fraser River	100	15	Yukon River	59
3	Rivers Inlet	38	16	Unalakleet River	93
4	Skeena River	100	17	Kotzebue Sound	100
5	Davidson Inlet	62	18	Kamchatka Coast (55-56° N., 164-169° E.)	89
6	Traitors Cove	100	19	Kamchatka Coast (56-59° N., 166-169° E.)	49
7	Stikine River	100	20	Okhotsk Sea	77
8	Port Houghton	41	21	Ubetsu River	96
9	Taku River	95	22	Tokachi River	85
10	Cook Inlet	90	23	Ishikari River	21
11	Uyak Bay	100		Total	1,741
12	King Cove	100			
13	Bristol Bay	61			

FIGURE 6. "Shore" sampling locations for chum salmon utilized in 1956 scale studies.

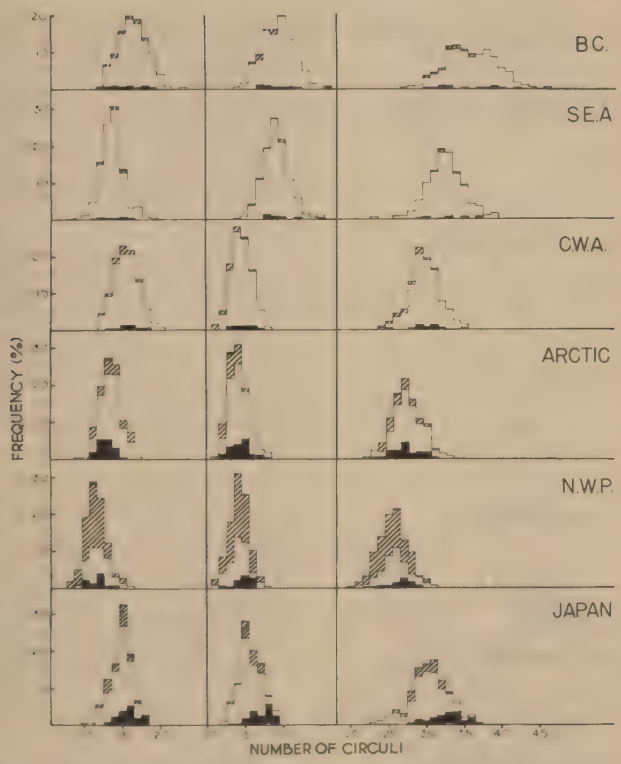


FIGURE 7. Distribution of first-year circulus counts for scales of chum salmon from different areas of the North Pacific.

Column A — Circuli in first half of first-year band.
Column B — Circuli in last half of first-year band.
Column C — Total first-year circuli.

Black bars indicate frequencies for 3$_1$ fish, white for 4$_1$'s and cross-hatched for 5$_1$'s.

While there were marked differences in the number of first-year circuli, there was relatively little difference in the width of the first annulus for fish originating in the different areas. The only group that tended to be divergent was that composed of fish sampled off the Siberian coast. The average width of the first annulus of these fish tended to be somewhat smaller than that for fish from the other 5 regions. (See Fig. 8).

B. Comparisons of Characters of High-seas Samples and "Shore" Samples. The scales of 2,010 chums taken on the high seas in 1956 were examined. For presentation of the data individual sampling stations have been grouped to provide 8 general areas, which are shown in Figure 9, along with the "shore" sample areas described in the previous section. In considering the high-seas data, differences in the time of sampling must be considered. The mid-Pacific samples (from Areas 5 and 6 in Fig. 9) were obtained early in the season (between May 30 and July 7). Samples in the Gulf of Alaska (from Areas 1, 2 and 3) and in the Western Pacific (from Areas 7 and 8) were obtained in

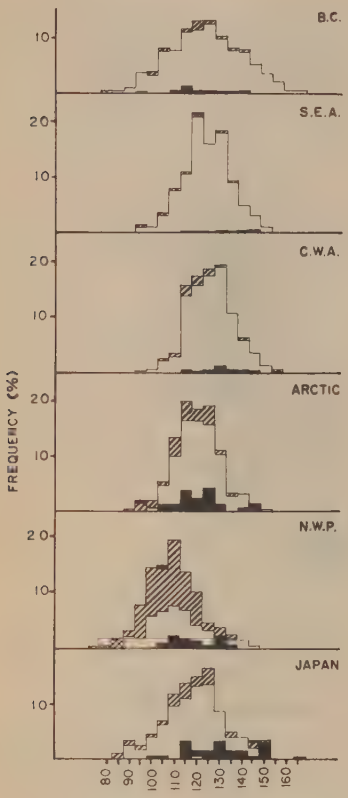


FIGURE 8. Width of first-year band in scales of chum salmon from different areas of the North Pacific.

mid-summer (July 2 to August 7). Samples from Area 4, between 160° and 170°W., south of the Aleutians were obtained later in the season (between August 18 and Septembr 13).

Numbers of circuli in whole and in last half of first-year zone. The number of circuli for the first year of life for fish from the 7 "shore" areas and 8 high-seas sampling areas are illustrated in Figure 9. Data for the "shore" samples are illustrated by the black histograms, those for the high-seas stations by the clear histograms.

The data indicate that circulus counts for fish taken from all high-seas stations westward from 175°W., in both the early and middle parts of the season were consistently low, averaging between 24.1 and 25.7. These values were similar to those for samples taken in the North Bering and in the Sea of Okhotsk close to the Kamchatka coast and were distinctly lower than averages for any "shore" sample on the North American side or for Japan (averages for North American "shore" samples ranged from 27.3 to 35.1, the Japanese samples averaged 30.4). Over half of the fish taken in western high-seas areas had circulus counts of 25 or less; only a few fish from spawning grounds in North America (excepting those of the "Arctic" area) and in Japan had circulus counts as low. These facts strongly suggest that the bulk of the fish taken in the area westward from 175°W. had not originated in the chum-producing areas of Alaska and B.C. nor in the rivers of Japan. Presumably, then, they had originated in the spawning streams of continental Asia, with the possibility that some might have come from northern Alaska. Data on the annulus widths presented later strengthen this view.

The average circulus counts for chums taken in the area eastward from 170°W. were consistently higher than those for fish taken in the western North Pacific area. The average first-year circulus counts for fish from the 4 high-seas areas eastward from 170°W. were quite similar to the average for fish from central and western Alaska. However, the frequency distributions of the circulus counts (Fig. 9) were characterized by wide ranges and flat domes suggesting that the samples were mixtures of fish of different origins.

Examination of the number of circuli in the last half of the first-year band provides more precise information on the probable origin of fish contributing to these mixed samples. As indicated in Figure 7, fish having 20 or more "last-half" circuli were found only among "shore" samples from the B.C.-southeast Alaska area. Last-half circulus counts for fish both from "shore" areas and from the 8 high-seas fishing areas are shown in Figure 10. In Table 8 the percentages of fish exhibiting counts of 20 and more from each area are listed. The results indicate that fish having last-half circulus counts of 20 or more, which were presumed to be B.C.-southeast Alaska fish, were restricted almost entirely to the area eastward from 170°W. Of the 1,392 fish from both high-seas and "shore" samples taken in areas westward from 175°W., only one had a count of 20 or over. Thus, B.C.-southeast Alaska fish failed to form a significant fraction of the early and mid-season samples of chums taken in the Western Pacific.

Within the Gulf of Alaska area, where the fish with high circulus counts contributed significantly to the catches, the greatest proportion (23.9%)

TABLE 8. Number of fish having circulus counts for the last half of the first year band of 20 or greater for chum salmon sampled in 1956 from 7 "shore" areas and 8 high-seas sampling areas.

Sampling area	No. fish sampled	No. fish with 20+ circuli	
		No.	%
British Columbia	306	151	49.3
Southeast Alaska	398	155	38.9
Area 1	142	34	23.9
Area 2	300	2	.6
Area 3	301	22	7.3
Central and western Alaska	368	0	0
Area 4	292	32	10.9
Arctic	252	0	0
Area 5	117	0	0
Area 6	365	1	.3
NW Pacific	138	0	0
Area 7	181	0	0
Area 8	312	0	0
Okhotsk Sea	77	0	0
Japan	202	0	0

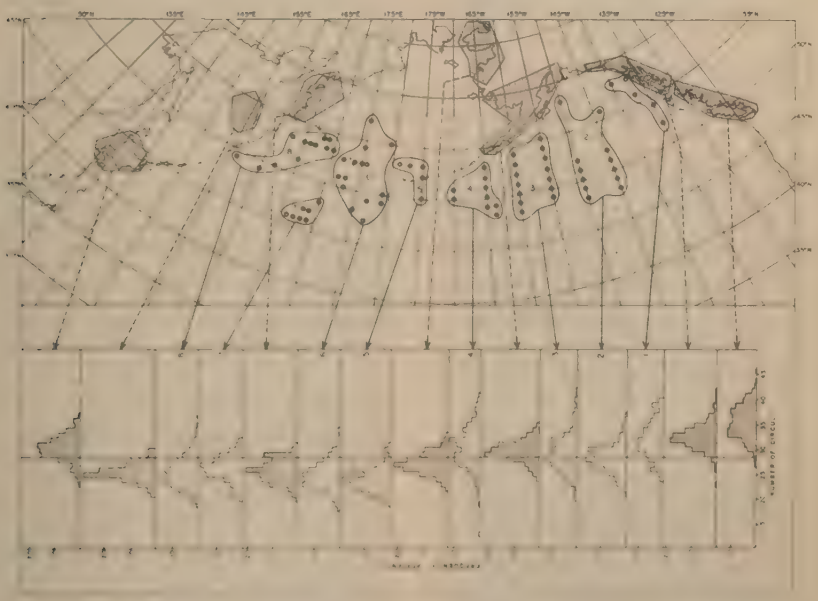


FIGURE 9. Total number of circuli in first-year band in scales of chum salmon from various areas in the North Pacific in 1956.

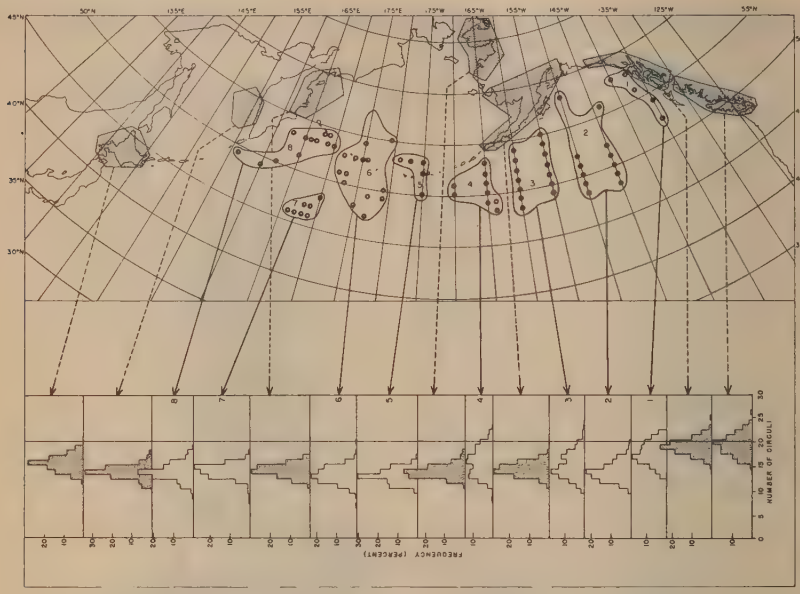


FIGURE 10. Number of circuli in the last half of the first-year band in scales of chum salmon from various areas in the North Pacific in 1956.

was found in the mid-season catches immediately offshore from the B.C.-southeast Alaska coast (Area 1). The mid-season and late-season samples from Areas 3 and 4 immediately south of the Alaska Peninsula also showed substantial numbers of high-count B.C.-southeast Alaska fish (7.3 and 10.9% respectively). However, the mid-summer sample from the central Gulf of Alaska (Area 2) contained only a small fraction of fish of the B.C.-southeast Alaska type (0.6%).

Width and number of circuli in first-year zone. Chum scales from the "shore" areas of the west Bering Sea and from the Sea of Okhotsk had few first-year circuli and narrow first-year zones. Table 9 shows the percentages of fish from each of 7 "shore" areas and 8 high-seas areas having first-year circulus counts of less than 24 *and* first-year zone widths of 1.15 mm. or less. Among the "shore" samples, those from the Kamchatka coast on the Bering Sea side contained the greatest number of these fish with low circulus count and low zone widths (averaging 25.4%). The Sea of Okhotsk samples had 11.7%, the Arctic Sea had just 2.0%, while all other "shore" areas had fewer than 1% of this type of fish.

Examination of the characteristics of the 1956 high-seas samples indicates that in the region westward from 175°W. (Areas 5 - 8), all samples contained substantial proportions of fish with first-year circulus counts of 24 or less and first-year zone widths of 1.15 mm. or less. In this region, between 15.5% and 48.7% of the fish sampled exhibited the low counts and small widths. Considering the fact that no "shore" area in North America exhibited samples with more than 2% of fish of this type, the great majority of fish in the area westward from 175°W. were most probably fish of continental Asiatic origin. Fish from Areas 5 and 6 in the central North Pacific Ocean and central Bering Sea had even higher proportions of "low count-small width" fish than did the "shore" samples used to characterize continental Asiatic stocks. One explanation for this might be that the so-called "shore" samples from the western region were not truly representative of chum stocks originating in Asian streams; it is possible that Asiatic populations exist which contain even higher proportions of "low count-small width" fish than those represented in the type samples taken off the Kamchatka coast.

TABLE 9. Numbers of chum salmon with first-year circulus counts of less than 24 and first-year zone widths of 1.15 mm. or less in samples from 7 "shore" areas and 8 high-seas areas in 1956.

Sampling area	No. fish sampled	No. fish with 24 - circuli 1.15 - annulus width	
		No.	%
British Columbia	306	--	--
Southeast Alaska	398	1	.2
Area 1	142	2	1.4
Area 2	300	21	7.0
Area 3	301	13	4.3
Central and western Alaska	368	1	.3
Area 4	292	10	3.4
Arctic	252	5	2.0
Area 5	117	57	48.7
Area 6	365	136	37.3
NW Pacific	138	35	25.4
Area 7	181	28	15.5
Area 8	312	89	28.5
Okhotsk Sea	77	9	11.7
Japan	202	1	.5

In the samples taken from the areas eastward from 170°W. (Areas 1 - 4), the proportion of "low count-small width" fish was higher than for the "shore" samples taken on the adjacent spawning streams. This was especially true for the mid-summer samples from the center of the Gulf of Alaska (Area 2) where 7% of the fish sampled possessed very low circulus counts and annulus widths. It is tentatively concluded that at least some of the fish caught in this area were of continental Asiatic origin.

Discussion. In interpreting the foregoing data, a number of limitations must be borne in mind. First, the "shore" samples used to characterize fisn originating in continental Asia were actually composed of fish taken in offshore fishing and may well have included fish bound for other areas. Samples of fish from the large chum-producing rivers of the U.S.S.R. are still required to provide a sound assessment of the continental origin of fish in the high-seas catches. Second, the present study must be extended to include data for other years. In examining the 1956 data, the "shore" samples for fish from North American and Japanese rivers were composed mainly of 4-year-olds while 5-year-olds predominated in the Northwest Pacific and Okhotsk samples. However, the high-seas samples studied contained substantial numbers of 2- and 3-year-olds. For this reason, data for "shore" samples in succeeding years would be desirable for a more accurate interpretation of the results. Third, in the present analysis, there were not enough samples available from the important area in the vicinity of 175°W. It is hoped that as scales from collections of whole fish become available this shortcoming will be remedied.

Despite these deficiencies, this preliminary study did reveal marked differences in the characteristics of scales from chum salmon taken in various parts of the North Pacific. The following tentative conclusions are reached:

(1) The area westward from 175°W. On the basis of first-year circulus counts and measurements of annulus widths, high-seas samples of chum salmon taken in the area westward from 175°W. in 1956 did not appear to contain an appreciable proportion of fish originating in North American streams south of the 62°N. parallel. Fish of the type found in Japanese streams apparently did not form a substantial fraction of the total population sampled in this area. Similarities in the types of scales found on fish from the northernmost part of Alaska and from fish taken close to Kamchatka indicate that some fish in the western half of the North Pacific might have originated in the northernmost chum streams of North America. It is concluded, however, that the majority of chums taken on the high seas westward from 175°W. originated in Asiatic streams outside of Japan.

(2) The area eastward from 170°W. High-seas samples examined from the area eastward from 170°W. were restricted mainly to the region south of the Aleutians and in the Gulf of Alaska. These samples contained fish which could be identified positively as originating in the streams of B.C. or southeast Alaska and a minor fraction which could be ascribed to fish originating in continental Asia. The remainder, which formed the bulk of the samples, were fish with intermediate scale characteristics which could, in theory, have originated in the central area of Alaska or in Japan.

In view of the proximity of the sampling areas to the important spawning grounds of Alaska and of the lack of substantial proportions of fish of the Japanese "shore" sample type in the high-seas catches of the central Pacific, it is concluded that most of the unclassified fish were North American in origin.

4. OCEANOGRAPHY

Since the last summary report (1957) three surveys have been completed. The first, essentially a winter survey, was from March 11 to April 10. The second, a summer survey, was from July 22 to August 16. These covered the Gulf of Alaska and adjacent ocean. The third survey was carried out between June 27 and August 14, during which time one month (July 5 to August 5) was spent in the vicinity of the Aleutian Islands. Observations were also made enroute to and from this area and have been incorporated with those in the eastern Pacific. Observations at ocean weather station "P" (latitude 50°N., longitude 145°W.) have continued regularly, through alternate six-week periods.

These data have been published. They have been supplemented by data from the Department of Oceanography, University of Washington, Seattle, and the United States and Canadian exploratory fishing vessels. Some of the important oceanographic features noted in these data are briefly summarized.

March, 1958 — Gulf of Alaska

Salinity. The horizontal distribution of salinity at 10 meters is shown in Figure 11. The configuration of the isohalines is generally the same

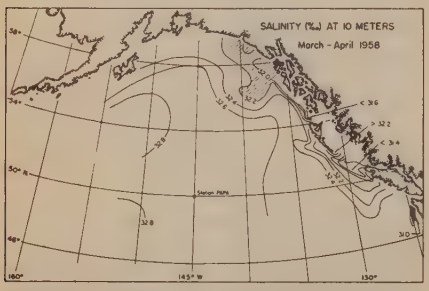


FIGURE 11. Salinity at 10 meters depth in the eastern North Pacific in March-April, 1958.

as for the previous summer. There was a salinity maximum (>32.8‰) in the Gulf of Alaska, which is associated with the anti-clockwise circulation around this centre. Northward and eastward of the centre the salinity decreased. Southward the salinity decreased then increased, similar to former periods. One feature that differs from the previous period was the extension of low salinity water (<32.2‰) offshore off the eastern side of the Gulf of Alaska.

The salinity distribution at 100 meters depth (Fig. 12) is generally representative of the distribution of fresh water in the upper zone—i.e. low salinity values indicate a large accumulation of

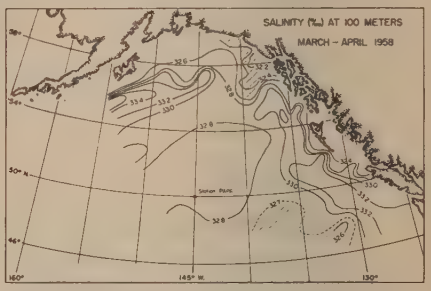


FIGURE 12. Salinity at 100 meters depth in the eastern North Pacific in March-April, 1958.

fresh water in the upper zone. Again the configuration of the isohalines is generally similar to the previous summer, and somewhat similar to the previous winter. However, in the eastern Gulf of Alaska, there was an area of low salinity water (<32.4‰). This indicates a much larger accumulation of fresh water in this area than in previous periods.

The evident cell of relatively low salinity (<32.7‰), between longitudes 130° and 140°W., off the Washington-Vancouver Island coast, present in previous surveys, is also indicated.

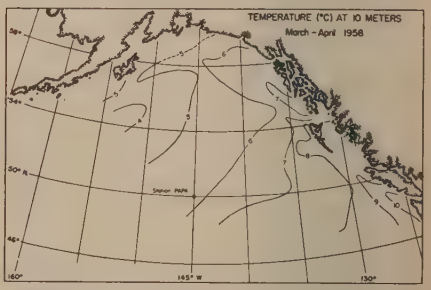


FIGURE 13. Temperature at 10 meters depth in the eastern North Pacific in March-April, 1958.

Temperature. As in the previous winter the cold centre (<4°C.) in the Gulf of Alaska (Fig. 13) corresponded in position with the centre of high salinity. Compared to the previous winter there was a more northward slant of the isotherms between ocean weather station "P" and the coast. Also the 4°C. isotherm enclosed a smaller area than in the previous winter. Thus, over the whole area, surface temperatures were about 1 to 2 degrees warmer than during the previous winter.

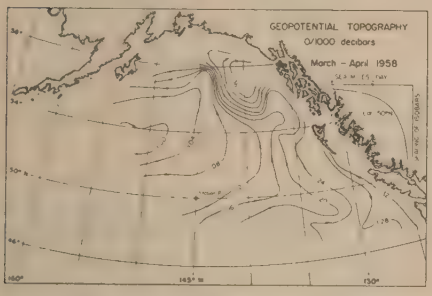


FIGURE 14. Geopotential topography in the eastern North Pacific in March-April, 1958.

Calculated currents. Figure 14 shows the geostrophic flow for this period. The general features of the circulation persisted. There are two marked differences from the previous summer. The flow was more northerly in the mid-Gulf area during this period than previously. This suggests intrusion of southern-type water into the area and would account for the high surface temperatures off the Canadian coast. Secondly, associated with the low salinity centre in the eastern Gulf of Alaska, there was a clockwise gyral.

August, 1958 — Gulf of Alaska

Salinity. As shown in Figure 15, the salinity at a depth of 10 meters was least in the shallow water over the continental shelf and increased seaward. The low salinity water appears to extend farther offshore than previously, especially in the northern part of the Gulf of Alaska. Also the high salinity centre in the Alaska Gyral was not evident.

The salinity distribution at 100 meters depth is presented in Figure 16. Relatively high values were observed along the west coast of Vancouver Island and in the vicinity of the Alaska Gyral. These are typical, although in the former region the values are perhaps higher than usual. An interesting feature of the figure is the indication of clouds of low salinity water (<32.7°/$_{00}$) (shaded) lying well seaward off the mainland coast.

FIGURE 15. Salinity at 10 meters depth in the eastern North Pacific in July-August, 1958.

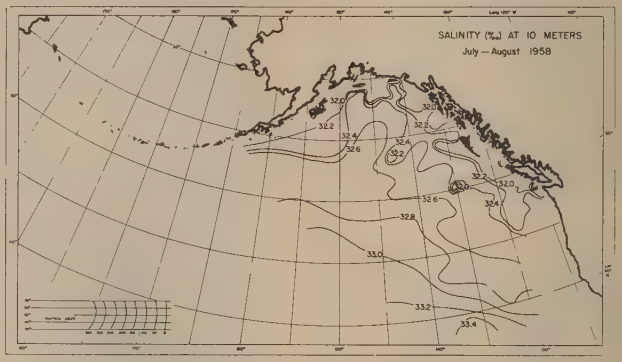


FIGURE 16. Salinity at 100 meters depth in the eastern North Pacific in July-August, 1958.

Temperature. The observed distribution of temperature at 10 meters depth is shown in Figure 17. A comparison with earlier reports suggests that in the oceanic region eastward of the longitude of station "P" the temperatures were not only above average, but also above those observed in the summer of 1957.

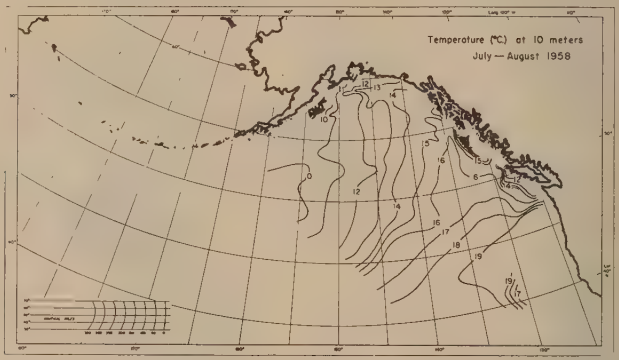


FIGURE 17. Temperature at 10 meters depth in the eastern North Pacific in July-August, 1958.

Calculated currents. The geopotential topography referred to the 1,000 decibar surface is shown in Figure 18. The general configuration of the isobars suggests the existence of a somewhat different situation from that observed in the previous surveys. The isobars in mid-ocean exhibit a stronger than usual north-south tendency, and in addition, there is evidence of a southerly movement in the water adjacent to the Canadian coast. This latter feature is perhaps the most interesting since it represents a marked departure from what is considered the usual. It was, however, observed in August, 1936.

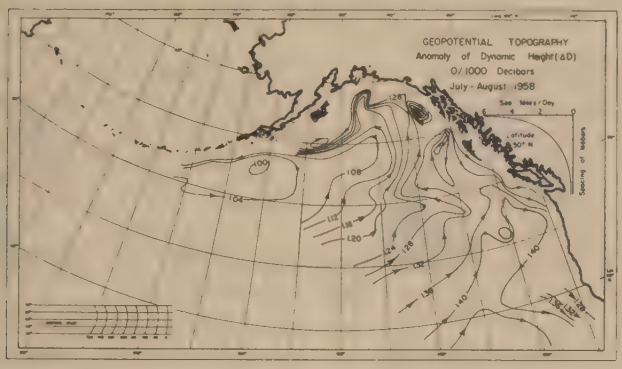


FIGURE 18. Geopotential topography in the eastern North Pacific in July-August, 1958.

General Summary of Conditions in the Gulf of Alaska. Between Station "P" and the coast the movement of water has veered progressively northward since 1955. Associated with this there has been a progressive increase in the temperature of the surface waters in this region from summer to summer and winter to winter.

Further analysis shows that this increase was not solely a result of seasonal heating and cooling and thus limited to the depth of seasonal influence (125 meters). That the warming extended into the non-seasonal waters can be shown by considering the temperature distribution on the surface at which the density is 1.0266 ("sigma-t"=26.60). Figure 19 shows that the depth of this surface was generally greater than 175 meters in the area off the Canadian coast and therefore below the depth of seasonal influence. Figure 20 shows the distribution of temperature on this surface for the 6 periods since 1955. In this analysis it is assumed that the flow in general is parallel to the isotherms, which is confirmed by the calculated currents. Thus the changes in circulation of the intermediate water from the summer of 1955 to 1958 are reflected in these figures. They show that there was a progressive northward intrusion, off the Canadian coast, of warm water, arbitrarily indicated by the water of temperature 6.5°C. or greater (shaded areas). The intrusion reached a maximum in March, 1958, and persisted until at least August, 1958. This suggests that the northerly offshore flow postulated from the summer data of 1957 was intensified during the winter period, hence

FIGURE 19. Depth of the surface at which the density is 1.0266.

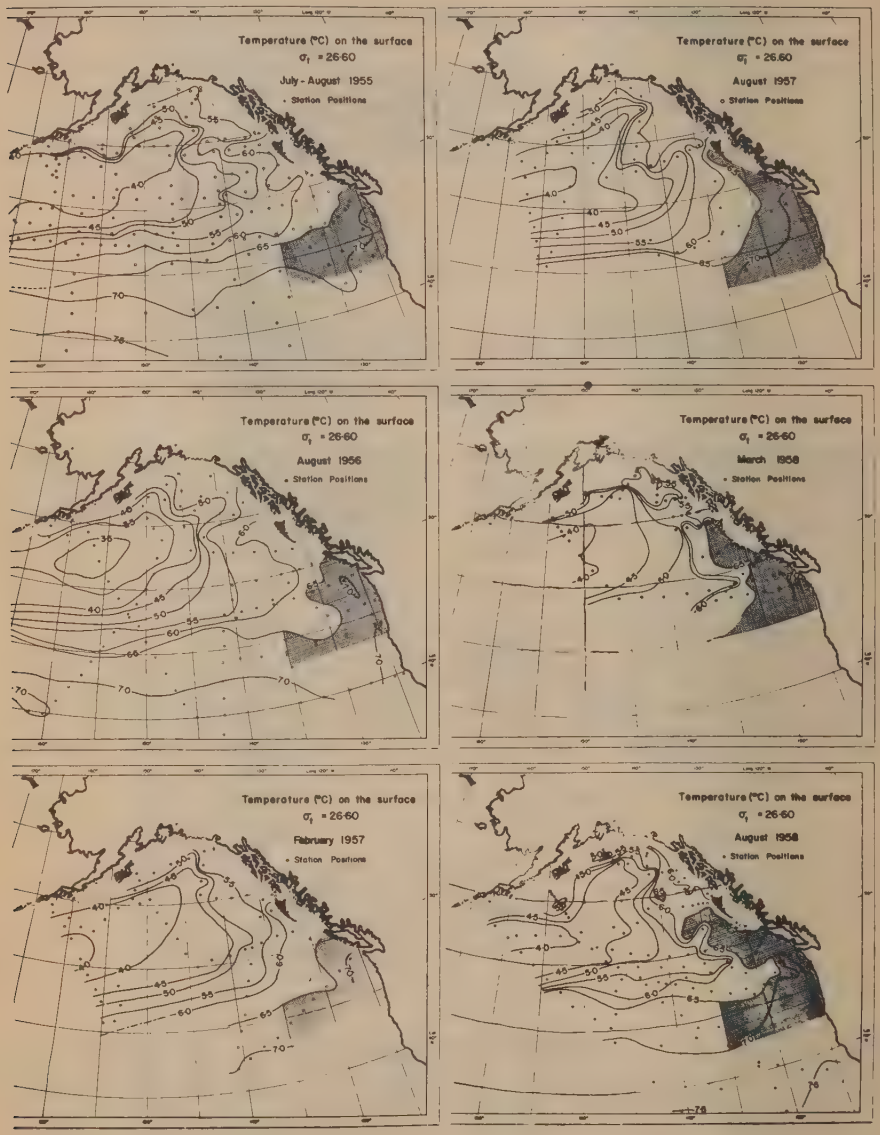


FIGURE 20. Temperatures on the surface of density 1.0266.

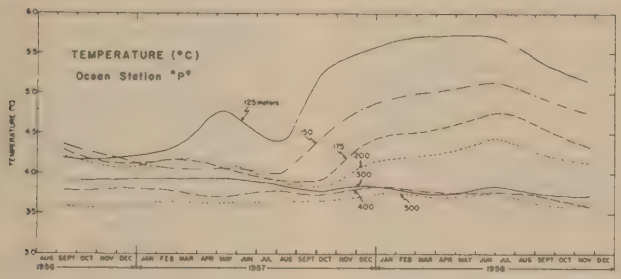


FIGURE 21. Temperature structure at Station "P"

making it possible for warm water from the south to flow into this region.

The warm water intrusion between Station "P" and the coast is reflected in the temperature structure at Station "P" down to a depth of 500 meters (Fig. 21). These data show that the warm water intrusion had intensified since August, 1957, and was at a maximum in July, 1958, at Station "P". More recent data suggests that the warm water intrusion is relaxing at Station "P". Thus it is possible that oceanographic conditions off the Canadian coast and extending to Station "P" may be similar to those observed in August, 1957, within the next two or three months. The winter survey in 1959 should confirm or disprove this.

Oceanographic Observations in the Aleutian Area, July-August, 1958. Figures 22 and 23 show the vertical distribution of temperature and salinity along 179°30'E. longitude. North and south of the Aleutian Islands there was a well-defined thermocline extending to about 60 meters depth. Within this layer higher salinity and slightly lower temperatures occurred in the Bering Sea than in the region south of the Islands. The temperature then decreased to a sub-surface minimum (dichothermal water); then increased to a maximum (mesothermal water). Associated with the mesothermal water, the salinity structure showed a halocline, or a gradient of increasing salinity sufficient to make the structure stable. Below this layer, the temperature gradually decreased and salinity increased with depth.

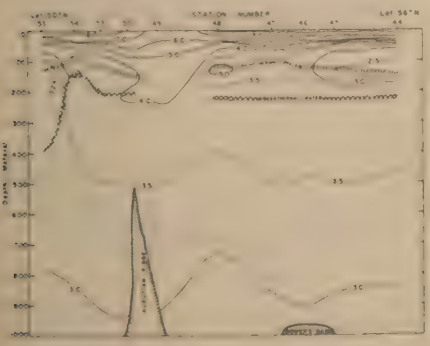


FIGURE 22. Temperature section along 179°30'E. Longitude, July 20-23, 1958

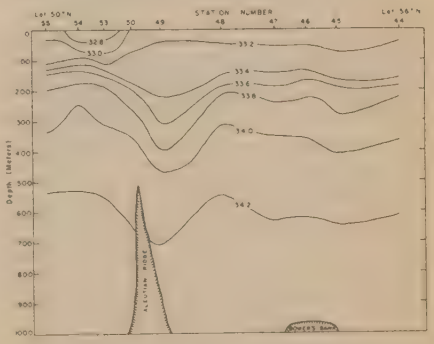


FIGURE 23. Salinity section along 179°30'E. Longitude, July 20-23, 1958.

The depth and temperature of the mesothermal water were fairly constant in the Bering Sea, except in the eastern part (Fig. 24). This was especially true in this section, in which its depth was approximately 220 meters and the temperature varied by only 0.17 degrees. South of the Islands both the depth and temperature of this water varied. This is noted particularly at Station 55 (Fig. 22), the southern limit of this section. At this station, the depth of the mesothermal water was about 400 meters, and the temperature was 3.4°C., approximately 200 meters deeper and 0.7 degrees

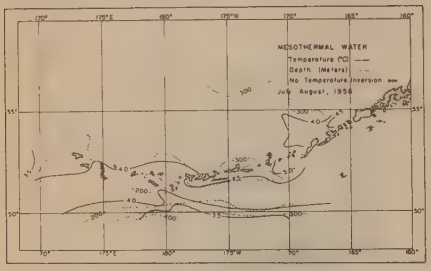


FIGURE 24. Temperature and depth of the mesothermal water in July-August, 1958.

colder than the water to the north. This suggests a marked discontinuity between Stations 54 and 55; a colder and less saline water mass in the depth interval 100 to 400 meters to the south than to the north. This feature is worthy of closer examination.

The temperature and depth distributions of the mesothermal water are shown in Figure 24. South of the Islands relatively warm water (>4.0° C.) extended westward to at least 170°E. longitude and intruded into the Bering Sea between longitudes 177°W. and 174°E. Similar warm water also appeared in the southeastern part of the Bering Sea. Over the remainder of the area, north of the Islands, the temperature of the mesothermal water was fairly constant and less than 4.0 degrees.

It is probable that the cold water mass (<3.5° C.) described at Station 55, extended eastward to the vicinity of longitude 170°W.

The temperature and depth distribution of the dichothermal water are shown in Figure 25. The depth of the water generally varied between 100 and 150 meters and was found at greater depths in the Bering Sea than south of the Islands.

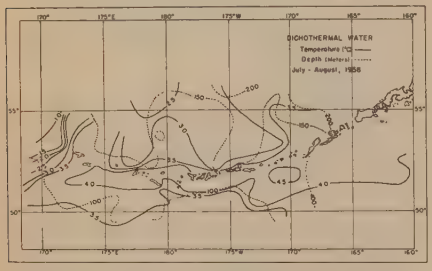


FIGURE 25. Temperature and depth of the dichothermal water in July-August, 1958.

South of the Islands the dichothermal water was relatively warm (>4.0° C.). This water showed much the same distribution as the warm mesothermal water with an intrusion into the Bering Sea. At the western and north-western limits of the survey, low temperatures were common (<2.5° C.). In the western area there was a marked gradient extending to the western tip of Attu. This suggests an area of convergence, probably having a seasonal and yearly shift. Northward of the Islands the temperature of the dichothermal water was generally less than 3.5 degrees.

A comparison of the temperatures of the mesothermal and dichothermal waters shows that generally in the Bering Sea there were marked differences in the temperatures of these waters. However, south of the Islands, the differences were small. In fact there were areas where no mesothermal water occurred.

Figures 26 and 27 show the distribution of temperature and salinity at 10 meters depth. North of the Aleutian Islands salinity was higher and temperatures were slightly lower than those south

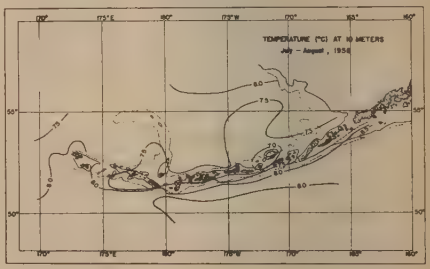


FIGURE 26. Temperature at 10 meters depth in July-August, 1958.

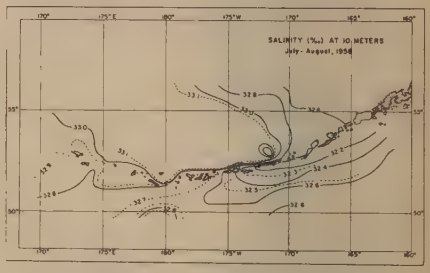


FIGURE 27. Salinity at 10 meters depth in July-August, 1958.

of the Islands. In the near vicinity of the Aleutian Islands there was generally a marked gradient. This is particularly noted in the salinity distribution (Fig. 27) westward to longitude 175°W.

The calculated currents are shown in Figure 28. Close along the southern side of the Aleutian

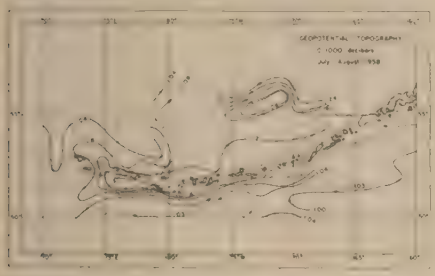


FIGURE 28. Geopotential topography in July-August, 1958.

Islands there was a relatively strong westerly flow, originating from the Gulf of Alaska, extending as far west as 173°E. longitude. There was a marked intrusion into the Bering Sea east of Attu, and then an easterly return flow close along the north side of the Aleutian Islands. The main flow in the Bering Sea seemed to conform to the bottom topography. In the western part, the flow followed around on the west side of Bowers Bank. The remainder of the flow continued eastward to the Continental Shelf where it again was deflected northward and then westward, probably by the configuration of the bottom topography.

South of the Aleutian Islands, in the vicinity of the cold mesothermal water (Fig. 24), it is suggested that the flow from the west approaches the Islands and results in an anti-clockwise circulation. This results in an interchange of water between these two streams.

Although not evident in this picture, there was a marked surface flow through the channels. In most cases this water lost its structure due to extreme mixing.

III. THE RESEARCH PROGRAM

A. PROGRESS IN 1959 IN CANADIAN RESEARCH ON PROBLEMS RAISED BY THE PROTOCOL

In 1959 Canada continued the following lines of research as part of the Commission's program: (a) high-seas fishing to study salmon distribution and obtain samples for study, (b) studies of parasites of sockeye salmon and of scales of chum salmon to distinguish stocks of various river origins and discover their distribution on the high seas and (c) study of the oceanography of the North Pacific to provide background for understanding salmon distribution and movements. Canada also continued the provision of samples for study by United States and Japan and efforts to recover tags placed on salmon on the high seas.

1. HIGH-SEAS FISHING TO STUDY SALMON DISTRIBUTION AND OBTAIN SAMPLES

Vessel operations and gear. Fishing was carried out by the same vessels as in 1958, namely the *Key West II* and the *Fort Ross*. *The Key West II* left Nanaimo on May 7 and returned from her final cruise on July 27. Corresponding dates for the *Fort Ross* were May 9 and August 11.

The fishing gear used by the *Key West II* consisted of surface gillnets of mesh size $2^1/_2$", $3^1/_4$", $4^1/_2$" and $5^1/_2$", in the proportion 1:1:3:1, as in 1957 and 1958. The amount of gear set at one time was either 36 or 35 50-fathom shackles.

The *Fort Ross* was engaged in investigating the vertical distribution of salmon. For this purpose she used a gillnet 400 fathoms long (reduced to 350 fathoms after mid-June) and 40 feet deep, of $4^1/_2$" mesh. This net could be suspended at any one of five depth positions, the series covering the interval from the surface to 200 feet. Additional surface gillnets, similar to those of the *Key West II*, were also carried.

Long-lines and deep-water trolls were carried by both vessels. Plankton nets and mid-water trawls of Isaacs-Kidd pattern were also used.

Collections of salmon for use in the morphometric, parasitological and scale studies of the INPFC program were made by the *Key West II* at seven stations, the localities of which are shown in Figure 1. From 1 to 3 night sets were made at each station between May 18 and June 30. After covering these stations a few sets of long-line and gillnet gear were made by the *Key West II* in waters adjacent to the southern end of the Queen Charlotte Islands.

The locations at which the depth gear was fished by the *Fort Ross* were: 50°N, 135°W; 55°N, 149°W; and an intermediate position which varied to some extent (Fig. 1). These stations were selected to provide different temperature conditions.

Distribution and age of salmon. Catches and accompanying data for surface gillnets fished at the sampling stations are shown in Table I. The relative size of catches of sockeye, pinks and chums, after weighting for the amount of gear and the length of time the gear was fished, is given in Table II. The ages of sockeye and chum salmon are shown in Tables III and IV. These tables supersede the preliminary determinations given in "Progress Report of Canadian Exploratory Fishing for Salmon in the Northeast Pacific in 1959" (INPFC Doc. 292).

Distribution of immature and maturing sockeye and chum salmon in the Gulf of Alaska. Fish caught by Canadian research vessels in 1957, 1958 and 1959 were classified on the basis of weight of gonads in relation to total body weight (percent) and age. A satisfactory division into "immature" and "maturing" categories was obtained by separating the fish which showed higher or lower index values than the following:

Species	Age	Sex	Index value
Sockeye	3_2	Male	0.2
Sockeye	4_2, 5_2, 5_3	Male	0.1
Sockeye	4_2, 5_2, 5_3	Female	1.0
Chum	3, 4	Male	0.1
Chum	3, 4	Female	1.0

Distribution of immature and maturing fish showed a consistent pattern throughout the three years, insofar as the locations and dates of sampling stations permitted comparison. During May and June both categories of sockeye were present through-

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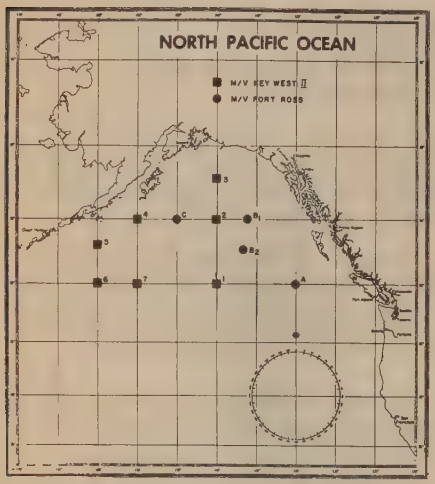


Figure 1. Sampling stations occupied by Canadian research vessels, 1959.

out the Gulf but immature fish were especially prevalent along Latitude 50°N, west of Longitude 145°W, constituting up to 90% of the sockeye caught in this area. They constituted a much smaller proportion of catches made at stations closer to land. In July and August maturing fish were virtually absent from catches made west of Longitude 145°W. Chum salmon showed a generally similar pattern but some maturing fish continued to occur in western areas until a later date than sockeye. The area near Latitude 50°N, west of Longitude 145°W appears to be an important feeding area for immature fish of both species.

Vertical distribution of salmon. In the investigations of the vertical distribution of salmon, the sunken net was fished in conjunction with a surface gillnet similar to those used in ordinary sampling operations but consisting of only 6 shackles. The catches made by each kind of gear are recorded in Table V. Relative numbers of sockeyes, pinks and chums caught at different depths, by time periods, are shown in Figure 2 and Table VI. These comparisons are limited to fish caught at

Table I. Salmon catches by the *Key West II*, 1959.

Date		Position		No. of shackles	Surf. temp.	Salmon				
		Lat. (°N)	Long. (°W)		(°C)	Sockeye	Pink	Chum	Coho	Total
May	18	50°00′	145°00′	36	7.9	46	9	19	—	74
	19	50°00′	145°00′	36	7.9	58	29	6	8	101
	21	55°00′	145°00′	36	7.0	130	7	30	—	167
	22	55°00′	145°00′	36	6.5	26	—	6	—	32
	23	55°00′	145°00′	36	6.8	156	2	9	—	167
	25	58°00′	145°00′	36	7.9	190	—	27	—	217
	26	58°00′	145°00′	?	8.6	13	—	3	—	16
June	10	54°56′	155°00′	36	8.1	105	5	26	—	136
	12	55°11′	155°00′	36	8.9	363	14	163	—	540
	14	53°00′	160°00′	36	8.1	14	76	27	—	117
	24	53°00′	160°00′	35	9.6	41	46	11	—	98
	25	53°00′	160°00′	35	9.4	104	148	60	—	312
	27	50°00′	160°00′	35	9.9	91	54	239	—	384
	28	50.00′	160°00′	35	10.0	129	89	—	—	218
	29	50°00′	155°00′	35	10.4	217	16	137	3	373
July	17	51°00′	130°15′	35	13.8	11	—	—	—	11
	18	51°00′	132°00′	35	13.5	4	—	—	—	4
	19	52°10′	131°24′	35	12.9	8	16	—	—	24
	24	52°00′	134°00′	35	13.1	3	—	—	—	3
	25	51°00′	132°00′	35	13.1	40	2	1	—	43
Total						1749	513	764	11	3037
Percent						57.6	16.9	25.1	0.4	100.0

Table II. Relative abundance of salmon. Catches are weighted to 900 fathoms of surface gillnet fishing 10 hours, each mesh size equally represented.

Station	Position Lat. (°N)	Long. (°W)	No. of sets	Weighted catch Sockeye	Pink	Chum
1	50	145	2	14	6	3
2	55	145	3	31	1	5
3	58	145	1	48	—	9
4	55	155	2	100	3	43
5	53	160	3	28	21	9
6	50	160	2	41	16	30
7	50	155	1	240	6	57

night, since the number of daytime sets was small. It is possible that diurnal vertical movements by the fish may complicate the picture.

In May, sockeye and chum salmon were caught at each of the five depth intervals but sockeye were more frequent in the top 40 feet and chums below 80 feet. Later in the season all sockeye and pinks and most of the chums were taken within 40 feet of the surface. The balance of the chums was between 40 and 80 feet. The apparent change in vertical distribution during the course of the season coincided with the development of a thermocline below the level at which fish were caught (Fig. 2).

Table III. Age composition of sockeye salmon catches in the northeast Pacific, 1959, excluding catches by *Key West* during July 17-25.

Date	Stn.	Age 3_2	4_2	4_3	5_2	5_3	5_4	6_2	6_3	6_4	7_4	Unknown	Total
						Key West II							
May 18–19	1	11	47	1	18	15	—	1	5	—	—	6	104
21–23	2	8	44	2	43	23	—	—	22	2	3	20	167
25–26	3	1	68	—	61	39	—	1	20	4	3	6	203
June 10–12	4	16	108	3	22	56	1	—	16	4	2	35	263
14–25	5	42	30	8	18	34	—	—	13	4	—	10	159
27–28	6	69	21	24	3	11	1	—	1	1	—	10	141
29	7	126	10	7	—	5	—	—	—	—	—	13	161
Total		273	328	45	165	183	2	2	77	15	8	100	1198
						Fort Ross							
May 17–20	B	7	13	—	32	8	—	2	10	1	—	3	76
26–28	C	—	10	—	3	6	—	—	3	—	—	1	23
June 10–12	C	—	12	—	4	10	—	—	1	—	—	—	27
July 5–11	A	—	8	—	7	2	—	—	1	3	—	1	22
15–21	B	1	16	—	2	1	—	1	—	—	—	—	21
25–Aug 1	C	5	8	4	—	4	—	—	—	1	—	2	24
Total		13	67	4	48	31	—	3	15	5	—	7	193

Table IV. Ages of chum salmon caught at Canadian sampling stations, 1959.

Date	Station	Age 2	3	4	5	Unknown	Total
May 18–19	1	1	16	3	0	5	25
21–23	2	0	11	34	0	0	45
25–26	3	0	4	21	4	1	30
Jun 10–12	4	0	74	67	2	46	189
14–25	5	1	59	26	0	12	98
27–28	6	0	62	95	4	78	239
29	7	0	75	29	1	32	137
Total		2	301	275	11	174	763

Plankton. Plankton samples taken in conjunction with fishing operations have not yet been thoroughly examined. As in 1958, the chaetognath *Sagitta lyra*, which is tentatively regarded as an indicator of warm water intrusion and possibly of conditions less favourable for feeding salmon, was widespread in the eastern part of the Gulf of Alaska.

2. STUDY OF SOCKEYE SALMON PARASITES TO DISTINGUISH STOCKS

During the past year the studies commenced in 1955 on the use of parasites of sockeye salmon (*Oncorhynchus nerka*) as a means of tracing the ocean distribution of North American and Asian

Table V. Salmon catches by the M/V *Fort Ross*, 1959.

Date		Position Lat. (°N)	Position Long. (°W)	No. hrs. fished	Surface temp. (°C)	Surface gill-net Sockeye	Pink	Chum	Coho	Total	Sunken gill-net Depth	Sockeye	Pink	Chum	Coho	Total
May	13[1]	50°00′	135°00′	8.0	8.5	—	—	—	—	—	0′– 40′	—	—	—	—	—
	17	55°00′	141°00′	8.5	6.4	6	—	3	—	9	0′– 40′	6	—	—	—	6
	17[1]	55°00′	141°00′	7.0	6.8	2	—	—	—	2	0′– 40′	1	—	—	—	1
	18	55°00′	141°00′	8.0	6.4	17	—	—	—	17	40′– 80′	2	—	—	—	2
	18[1]	55°00′	141°00′	7.0	6.6	5	—	—	—	5	40′– 80′	—	—	—	—	—
	19	55°00′	141°00′	9.0	—	15	1	—	—	16	80′–120′	—	—	5	—	5
	19[1]	55°00′	141°00′	6.0	6.8	2	—	—	—	2	80′–120′	2	—	—	—	2
	20	55°00′	141°00′	10.0	6.4	1	—	2	—	3	120′–160′	—	—	3	—	3
	20[1]	55°00′	141°00′	6.0	7.0	6	—	3	—	9	120′–160′	1	—	1	—	2
	21	55°00′	141°00′	8.0	6.6	22	—	21	—	43	160′–200′	1	—	2	—	3
	25	55°00′	150°00′	8.5	6.2		—not fished—				0′– 40′	4	2	1	—	7
	25[1]	55°00′	150°00′	7.5	6.8		—not fished—				0′– 40′	—	—	—	—	—
	26	55°00′	150°00′	8.5	6.5	7	1	1	—	9	40′– 80′	1	2	2	—	5
	26[1]	55°00′	150°00′	8.0	7.5	2	—	1	—	3	40′– 80′	1	—	6	—	7
	27	55°03′	149°54′	13.5	—	7	—	—	—	7	80′–120′	3	—	2	—	5
	28	55°03′	149°54′	8.0	6.6	7	—	—	—	7	120′–160′	—	—	—	—	—
	28[1]	55°03′	149°54′	5.0	7.2	—	—	1	—	1	120′–160′	5	—	1	—	6
June	10	55°39′	150°23′	9.0	8.8	13	—	4	—	17	0′– 40′	16	2	1	1	20
	11	55°39′	150°23′	9.0	9.1	6	4	—	1	11	40′– 80′	3	6	1	—	10
	12	55°39′	150°23′	7.0	9.6	8	1	—	—	9	80′ 120′	—	1	—	—	1
	13	55°39′	150°23′	—	9.5						—nets tangled—					
July	5	50°00′	135°00′	8.0	11.6	—	—	—	—	—	0′– 40′[2]	6	1	—	—	7
	6	50°00′	135°00′	8.5	11.8	2	—	—	—	2	40′– 80′	—	—	—	1	1
	7	50°00′	135°00′	8.5	12.0	4	—	—	—	4	80′–120′	—	—	—	—	—
	10	50°00′	135°00′	9.0	11.8	5	1	—	1	7	120′–160′	—	—	—	—	—
	11	50°00′	135°00′	9.5	12.0	11	—	—	—	11	160′–200′	—	—	—	—	—
	15	52°30′	142°00′	8.0	9.7	6	6	7	—	19	0′– 40′	3	11	6	—	20
	18	52°30′	142°00′	8.0	10.8	3	2	1	—	6	40′– 80′	—	—	2	—	2
	19	52°30′	142°00′	8.5	11.0	9	3	2	—	14	80′–120′	—	—	—	—	—
	20	52°30′	142°00′	8.5	11.1	1	1	6	—	8	120′–160′	—	—	—	—	—
	21	52°30′	142°00′	8.5	11.0	2	3	5	—	10	160′–200′	—	—	—	—	—
	25	54°10′	150°30′	8.0	10.7	10	3	2	—	15	0′– 40′	13	14	24	1	52
	26	54°10′	150°30′	8.0	10.8	11	3	27	—	41	80′–120′	—	—	—	—	—
Aug	1	54°30′	150°37′	8.0	11.2	3	1	1	—	5	40′– 80′	—	—	6	—	6
	2	54°30′	150°30′	20.0	11.1	—species unidentified—				5	160′–200′	—tangled; no catch—				
Total						193	30	87	2	312		68	39	63	3	173

[1] Daytime set.

[2] Length of net reduced from 400 fathoms to 350 fathoms from this date.

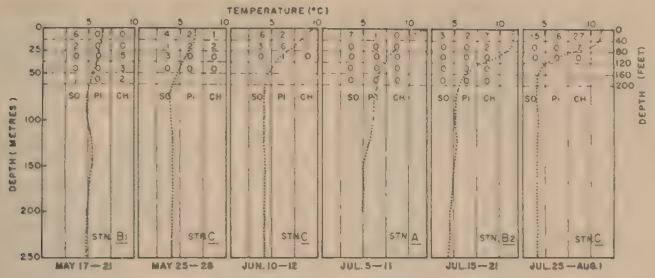


Figure 2. Average temperature profiles (dotted lines) at fishing stations with salmon catches (weighted) at various depths.

stocks of these fish were continued and expanded. Progress in the research for the years 1955 to 1957 has been reported in the Annual Reports for 1956, 1957 and 1958. The last report contained a review of the pertinent data for the three years during which the studies had been in progress.

In the Annual Report for 1958 four parasites were reported as providing evidence of the origin of sockeye taken on the high seas. Two of these were *Triaenophorus crassus*, a cestode found only in some western Alaska (mainly Bristol Bay) sockeye stocks, and *Dacnitis truttae*, a nematode found in some sockeye of Asian origin, both of which are parasites acquired in fresh water before the young sockeye migrate to sea. Once the fish take up their ocean residence further acquisition of these parasites becomes impossible. Since these parasites have a restricted geographical distribution in sockeye stocks they serve to identify the geographical origin of ocean-caught sockeye infected with them.

The other two parasites, *Tubulovesicula lindbergi* and *Hemiurus levinseni*, are trematodes acquired in the sea. *Tubulovesicula* was considered to indicate North American origin of sockeye infected with this species because of the characteristics of the distribution of sockeye infected with it and because it never occurred together with *Dacnitis* or *Hermiurus* in the same fish but was present in some sockeye simultaneously infected with *Triaenophorus*. The occurrence of *Hermiurus* in high-seas samples of sockeye, other than from the Gulf of Alaska, was suggested as indicating the Asian origin of such fish because of the characteristics of the distribution of sockeye infected with it and because it never occurred along with *Triaenophorus* or *Tubulovesicula* in the same fish,

Table VI. Relative numbers of sockeye, pink and chum salmon at various depth intervals to 200 feet. Data are based on sunken net catches at night weighted to 400 fathoms of gear.

Date	No. sets	Depth (ft)	Sockeye Av. catch per set	Sockeye %	Pink Av. catch per set	Pink %	Chum Av. catch per set	Chum %
May-June	3	0- 40	8.7	68.4	1.3	30.3	0.7	10.1
	3	40- 80	2.0	15.8	2.7	62.6	1.0	14.5
	3	80-120	1.0	7.9	0.3	7.1	1.7	24.6
	2	120-160	0.0	0.0	0.0	0.0	1.5	21.8
	1	160-200	1.0	7.9	0.0	0.0	2.0	29.0
Total			12.7	100.0	4.3	100.0	6.9	100.0
July-Aug.	3	0- 40	8.3	100.0	9.6	100.0	11.4	79.0
	3	40- 80	0.0	0.0	0.0	0.0	3.0	21.0
	3	80-120	0.0	0.0	0.0	0.0	—	0.0
	2	120-160	0.0	0.0	0.0	0.0	—	0.0
	2	160-200	0.0	0.0	0.0	0.0	—	0.0
Total			8.3	100.0	9.6	100.0	14.4	100.0

but was found in some fish also parasitized with *Dacnitis*.

Although in 1958 the distribution of fish infected with *Tubulovesicula* or *Hemiurus* fitted the general pattern of previous years, one sockeye was encountered which was infected simultaneously with *Hemiurus* and *Triaenophorus*, and another carried both *Hemiurus* and *Tubulovesicula*. The sockeye infected with *Triaenophorus* and *Hemiurus* was a maturing female, age 4_2, taken in the Bering Sea at 56°N, 172°30'W, and the sockeye infected with *Hemiurus* and *Tubulovesicula* was an immature male, age 4_3, taken south of the Aleutians at 50°N, 180°.

Triaenophorus indicates western Alaskan origin of any sockeye infected with it and therefore the occurrence of *Hemiurus* along with *Triaenophorus* in a maturing sockeye demonstrates that *Hemiurus* may be found in sockeye which are migrating to western Alaska. Consequently it can no longer be stated that every high-seas sockeye carrying *Hemiurus* (exclusive of those in the Gulf of Alaska) is of Asiatic origin. However, since we have rarely observed *Hemiurus* in sockeye samples taken near the shores or the mouths of rivers of western Alaska it is probably true that most ocean-caught sockeye infected with *Hemiurus* (except those in the Gulf of Alaska) are of Asiatic origin.

The occurrence of *Hemiurus* and *Tubulovesicula* in the same fish also conflicts with the use of these parasites in identifying the continent of origin of individual sockeye taken on the high seas. Although *Tubulovesicula* generally is associated with sockeye of North American origin, the suggestion that it is never found in sockeye of Asiatic origin may be doubtful, since the origin of the fish harbouring both *Hemiurus* and *Tubulovesicula* is unknown.

Since the parasites were used to identify the origin of individual fish it is apparent from the foregoing that neither *Hemiurus* nor *Tubulovesicula* can be considered to be completely reliable indicators. Some revision of previously presented conclusions (see Annual Report for 1958) on the distribution of sockeye stocks from North America and Asia are therefore necessary. The ensuing discussion on ocean distribution of sockeye from North America and Asia will be based only on the distribution of sockeye infected with *Triaenophorus* and *Dacnitis* and is applicable only within the limits of time and locations of the sampling.

Of all North American sockeye stocks, the ocean distribution of those from some western Alaskan rivers only can be traced with certainty, since it is in this region alone that *Triaenophorus* occurs in sockeye.

The Asiatic rivers in which the sockeye are infected with *Dacnitis* are not well known because of the lack of samples from Asian rivers. However, A. K. Akhmerov, in papers published in 1954 and 1955, recorded *Dacnitis* from Kamchatka River sockeye, and since we have consistently found *Dacnitis* in some of the sockeye from the Sea of Okhotsk (off the southwest tip of Kamchatka) it would appear that *Dacnitis* also occurs in sockeye from one or more rivers on the west coast of Kamchatka. *Dacnitis* therefore gives evidence of ocean distribution of some sockeye originating from both east and west Kamchatka.

In the laboratory 3,323 sockeye were examined from collections made in 1958, compared to a combined total of 4,610 for the three previous years, 1955 to 1957, inclusive. The 1958 samples consisted of 331 seaward migrants (smolts) from North America, 614 adults from coastal North America and 2,378 sockeye from the high seas. The high-seas samples, collected from mid-May to the latter half of August, included immature (fish which would have spent at least one more year at sea before returning to spawn) and maturing fish (fish which would have spawned in the year they were caught). The localities from which samples were collected, the date of each collection and the number of sockeye examined from each collection site are given in Tables VII to IX. Figure 3 also shows the distribution of coastal adult and high-seas sockeye samples.

A. **High-seas distribution of North American and Asiatic sockeye in 1958 as determined by *Triaenophorus* and *Dacnitis*.** In shore samples of adults from North America *Triaenophorus* was most prevalent in the Wood River (Nushagak River system) and was also found, but to a much lesser extent, in the Naknek River, both of which empty into Bristol Bay. It was also found in the seaward migrants from these two systems. *Triaenophorus* was not found in any other North American samples listed in Tables VII and VIII. In addition to the above two rivers, *Triaenophorus* was observed in previous years in one smolt from the Ugashik River, Bristol Bay and in one adult returning to spawn in the Kuskokwim River, on the west coast of Alaska, north of Bristol Bay.

Table VII. Locality, data of catch, and size of sockeye smolt samples examined for parasites from 1958 collections from North America.

Locality	Date of catch	Number of sockeyes
Baker R., Washington	20-VI	13
Cultus Lake, Fraser R., British Columbia	20-IV	25
Seton Creek, Fraser R., British Columbia	5 to 12-V	25
Chilko R., Fraser R., British Columbia	1-V	25
Babine Lake, Skeena R., British Columbia	7-VI	25
Sockeye Creek, Ketchikan, southeast Alaska		40
Karluk R., Kodiak Is., Alaska	24-VI	25
Chignik R., Alaska Peninsula	11-VI	25
Ugashik R., Bristol Bay, Alaska	27, 28-V	25
Brooks R., Bristol Bay, Alaska	25, 26, 27, 29-VI and 3-VII	28
Naknek R., Bristol Bay, Alaska	29-V	25
Kvichak R., Bristol Bay, Alaska	21, 22-V	25
Wood R., Bristol Bay, Alaska	31-V	25
Total		331

Table VIII. Locality, date of catch, and size of adult sockeye samples examined for parasites from 1958 collections from coastal North America. (The letters preceding the locality refer to the lettered positions in Fig. 3).

	Locality	Date of catch	Number of sockeyes
A.	Columbia R. (Astoria, Oregon)	22 to 28-VI	23
B.	Fraser R., British Columbia	6-VIII	25
	Fraser R., British Columbia	8-IX	25
C.	Rivers Inlet, British Columbia	13, 14-VII	25
D.	Skeena R., British Columbia	31-VII, 1-VIII	25
E.	Nass R., British Columbia	8-VII	25
F.	Naha Bay (Ketchikan), southeast Alaska	17-VII	25
G.	Stikine R. (Petersburg), southeast Alaska	9-VII	25
H.	Situk-Ahrnklin R. (Yakutat), Alaska	21-VII	25
I.	Eyak R. (Cordova), central Alaska	21-VII	25
J.	Seldovia, Cook Inlet, Alaska	14-VII	25
K.	Karluk R., Kodiak Is., Alaska	26-VI	25
	Karluk R., Kodiak Is., Alaska	31-VIII	24
L.	Red R., Kodiak Is., Alaska	24-VI	25
M.	Chignik, Alaska Peninsula	1-VII	25
N.	Shumagin Is., Alaska Peninsula	10-VII	25
O.	Ugashik R., Bristol Bay, Alaska	16-VII	25
P.	Egegik R., Bristol Bay, Alaska	7-VII	25
Q.	Naknek R., Bristol Bay, Alaska	9-VII	25
	Naknek R., Bristol Bay, Alaska	19-VII	27
R.	Kvichak R., Bristol Bay, Alaska	10-VII	25
S.	Wood R., Bristol Bay, Alaska	4-VII	25
T.	Togiak R., Bristol Bay, Alaska	12, 13-VII	25
U.	Kuskokwim R., northwestern Alaska	2, 4-VII	15
V.	Salmon Lake, northwestern Alaska	27-VII	25
	Total		614

Table IX. Locality, date of catch and size of high-seas sockeye samples examined for parasites from 1958 collections. (The numbers preceding the locality refer to the numbered positions in Fig. 3).

	Locality	Date of catch	Number of sockeyes
(1)	50°49′ N, 153°07′ E	5-VII	25
(2)	51°09′ N, 153°40′ E	25-VI	25
(3)	51°08′ N, 153°45′ E	15-VI	25
(4)	51°39′ N, 154°10′ E	15-VII	25
(5)	48°55′ N, 156°28′ E	5-VII	53
(6)	50°35′ N, 159°50′ E	19-VII	49
(7)	51°58′ N, 159°51′ E	26-VII	55
(8)	49°21′ N, 160°27′ E	16-VI	55
(9)	54° N, 163° E	2-VI	5
(10)	55°02′ N, 164°02′ E	4-VI	10
(11)	55° N, 164° E	7-VIII	50
(12)	55° N, 164° E	29-VII	50
(13)	56°01′ N, 164°58′ E	30-VI	5
(14)	58° N, 166° E	25-VI	6
	58° N, 166° E	26-VI	8
	58°02′ N, 166°03′ E	22-VI	4
(15)	49°35′ N, 166°35′ E	25-VI	54
(16)	59°01.5′ N, 167° E	21-VI	18
(17)	50°41′ N, 167°14′ E	15-VI	51
(18)	50°10′ N, 168°15′ E	16-V	54
(19)	59°16.5′ N, 169°03′ E	17-VI	20
(20)	47°30′ N, 169°41′ E	26-V	55
(21)	59°18′ N, 170°38′ E	31-VII	95
(22)	59°20′ N, 170°51′ E	13-VI	5
	59°28′ N, 170°54′ E	12-VI	3
	59°27′ N, 171°04′ E	11-VI	5
(23)	51°05′ N, 171°30′ E	29-V	53
(24)	52°02′ N, 171°35′ E	5-VI	53
(25)	53° N, 172° E	2-VI	12
(26)	50°50′ N, 172°30′ E	7-VI	55
(27)	51°35′ N, 173° E	8-VI	10
(28)	49°21′ N, 173°19′ E	16-V	54
(29)	54° N, 173° E	1-VI	17
(30)	55° N, 174° E	31-V	5
(31)	53°21′ N, 174°58′ E	22-VII	2
	54° N, 174°58′ E	21-VII	3
	55°01′ N, 174°58′ E	20-VII	2
	55°59′ N, 175°01′ E	19-VII	5
(32)	49° N, 175° E	15-VII	10
(33)	50° N, 175° E	16-VII	14
(34)	52° N, 175° E	18-VII	49
	53° N, 175° E	19-VII	3
(35)	54° N, 175° E	17-VII	6
	55° N, 175° E	18-VII	16
	54°02′ N, 176°28′ E	16-VII	2
(36)	50°37′ N, 175°02′ E	8-VI	11
(37)	49°39′ N, 176°11′ E	14-VII	4
	50°18′ N, 177°28′ E	13-VII	8
(38)	54° N, 178° E	15-VII	17
(39)	50°59′ N, 178°50′ E	12-VII	49
(40)	48°33′ N, 178°29′ E	1-VI	3
	49° N, 180°	31-V	7
	50°03′ N, 180°	29-V	8
	51° N, 180°	28-V	8
(41)	50° N, 180°	30-VI	42
(42)	53° N, 178°15′ E	29-V	5
	53° N, 180°	28-V	6
(43)	54°43′ N, 178°14′ E	22-VI	9
	53° N, 180°	17-VI	3
	54° N, 180°	18-VI	11
(44)	53°31′ N, 180°	14-VII	26
(45)	52°04′ N, 178°38′ W	12-VII	14
(46)	54° N, 178° W	10-VI	11
(47)	52°58′ N, 175°13′ W	27-VII	7
(48)	49°30′ N, 175° W	8-VII	4
	50°30′ N, 175° W	7-VII	15
	51°17′ N, 175°53′ W	6-VII	24
	50°22′ N, 177°45′ W	10-VII	5
(49)	53°01′ N, 175° W	2-VII	7
(50)	53° N, 174°59′ W	5-VIII	7
(51)	55° N, 175° W	7-VIII	13
(52)	51°30′ N, 175° W	5-VIII	53
(53)	50° N, 175° W	6-VIII	77
(54)	49° N, 175° W	7-VIII	27
(55)	56° N, 172°30′ W	18-VI	50
(56)	53°30′ N, 170°02′ W	17-VI	54
(57)	55°58′ N, 170°04′ W	26-VI	23
(58)	56°57′ N, 168°58′ W	27-VI	2
(59)	57°59′ N, 169°59′ W	28-VI	2
(60)	51° N, 170° W	11-VIII	52
(61)	50° N, 170° W	10 VIII	29
(62)	49° N, 170° W	9-VIII	11
(63)	55°04′ N, 169°24′ W	25-VI	79
(64)	52°51′ N, 166° W	15-VIII	24
(65)	52° N, 165° W	17-VIII	29
(66)	51° N, 165° W	18-VIII	27
(67)	50° N, 165° W	19-VIII	27
(68)	55° N, 155° W	15-VI	72
(69)	50° N, 155° W	25-VI	25
(70)	55° N, 150° W	7-VI	54
(71)	58° N, 145° W	4-VI	50
(72)	55° N, 145° W	1-VI	25
(73)	50° N, 145° W	24-V	25
(74)	55° N, 135° W	15-VI	16
(75)	55° N, 135° W	15-VIII	15
(76)	51° N, 130° W	8-VIII	25
	Total		2378

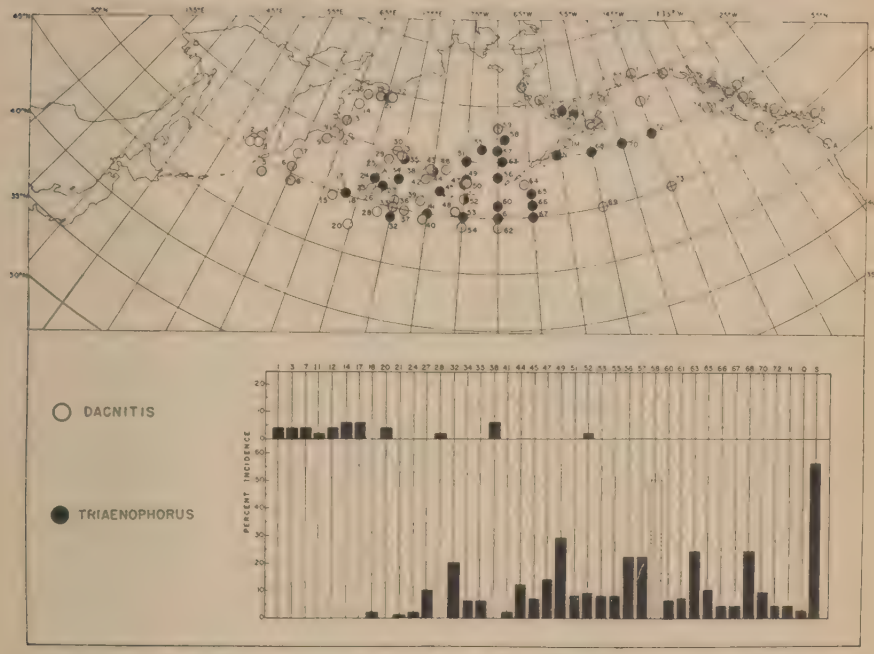


Figure 3. Distribution of sockeye salmon samples examined in 1958 and the occurrence and incidence of *Triaenophorus* and *Dacnitis* in these samples. (Stippled circles denote samples containing neither *Triaenophorus* or *Dacnitis*).

Shore samples from Asia were again unavailable but some samples taken close to the east coast of Kamchatka and some from the Sea of Okhotsk off the southwest coast of Kamchatka showed the presence of *Dacnitis*. It was not observed in any of the North American samples listed in Tables VII and VIII.

Figure 3 shows the distribution of sockeye (exclusive of seaward migrants) infected with *Triaenophorus* or *Dacnitis*. From this figure it is apparent that sockeye of western Alaskan origin (i.e. infected with *Triaenophorus*), mainly from Bristol Bay, were found as far west as 168°15'E and sockeye of Asiatic origin (i.e. infected with *Dacnitis*) were found as far east as 175°W. Since *Triaenophorus* and *Dacnitis* occur in only part of the sockeye originating in North America and Asia, respectively, and because of sampling limitations, the range of ocean distribution of sockeye from the two continents may be greater than indicated by these two parasites.

Table X lists the localities at which sockeye infected with *Triaenophorus* or *Dacnitis* were found, the date of catch, the number of fish examined at each locality, the number and percent of fish infected with *Triaenophorus* or *Dacnitis*, the age of these fish and whether they were maturing or immature. The determination of the state of maturity was based largely on the gonad development and utilized the gonad weight to body weight ratio, as proposed by Mr. H. Godfrey of the Fisheries Research Board's Biological Station at Nanaimo. In a few cases in which the gonad weight had not been recorded, the decision as to probable state of maturity was based on age, size of the fish, locality and date of catch.

Distribution of maturing sockeye. The upper chart in Figure 4 shows the distribution of maturing sockeye which harboured *Triaenophorus*

TAble X. Incidence of *Triaenophorus* and *Dacnitis* in 1958 sockeye samples, with age and stage of maturity of the infected fish.

Locality	Date	Number examined	Infected with *Triaenophorus* No.	%	Infected with *Dacnitis* No.	%	Age[a]	Maturity[b]
Wood R., Bristol Bay	4–VII	25	14	56	—	—	$4_2(10),5_3,5_2,?(2)$	m
" " " "	31–V	25	9	36	—	—	—	smolts
Naknek R., Bristol Bay	9, 19–VI	52	1	2	—	—	5_3	m
" " " "	29–V	25	1	4	—	—	—	smolts
55° N, 145° W	1–VI	25	1	4	—	—	4_2	m
55° N, 150° W	7–VI	54	5	9	—	—	$4_2(4),5_3$	m
55° N, 155° W	15–VI	72	17	24	—	—	$4_2(15),5_3,5_2$	m
Shumagin Is., Alaska	10–VII	25	1	4	—	—	5_3	m
50° N, 165° W	19–VIII	27	1	4	—	—	5_3	i
51° N, 165° W	18–VIII	27	1	4	—	—	4_2	i
52° N, 165° W	17–VIII	29	3	10	—	—	$4_2(2),5_3$	i
56°57′ N, 168°58′ W	27–VI	2	1	(50)	—	—	4_2	m
55°04′ N, 169°24′ W	25–VI	79	19	24	—	—	$4_2(9),5_3(3),5_2$ 2 oc. (5),?(1)	m
50° N, 170° W	10–VIII	29	2	7	—	—	$4_2,?(1)$	i
51° N, 170° W	11–VIII	52	3	6	—	—	$4_2,5_3,?(1)$	i
53°30′ N, 170°02′ W	17–VI	54	12	22	—	—	$4_2(4),5_3(2),5_2(5),6_3$	m
55°58′ N, 170°04′ W	26–VI	23	5	22	—	—	$4_2(3),5_3(2)$	m
56° N, 172°30′ W	18–VI	50	4	8	—	—	$4_2,5_3,5_2,6_3$	m
50° N, 175° W	6–VIII	77	6	8	—	—	$3_2,4_3,5_4,4_2,5_3(2)$	i
51°30′ N, 175° W	5–VIII	53	5	9	1	2	Triaen. $3_2,4_3$,2 oc.(2)	i
							Triaen. 4_2	m
							Dacnit. 3_2	i
53°01′ N, 175° W	2–VII	7	2	29	—	—	5_3 2 oc.	m i
55° N, 175° W	7–VIII	13	1	8	—	—	4_2	i
52°58′ N, 175°13′ W	27–VII	7	1	14	—	—	4_2	i
52°04′ N, 178°38′ W	12–VII	14	1	7	—	—	1 oc.	i
50° N, 180°	30–VI	42	1	2	—	—	4_3	i
53°31′ N, 180°	14–VII	26	3	12	—	—	$4_3,5_4,4_2$	i
54° N, 178° E	15–VII	17	—	—	1	6	3_2	i
49° N, 175° E	15–VII	10	2	20	—	—	$3_2,4_3$	i
52° N, 175° E	18–VII	49	3	6	—	—	$4_3(2),?(1)$	i
55° N, 175° E	18–VII	16	1	6	—	—	3_2	i
49°21′ N, 173°19′ E	16–V	54	—	—	1	2	5_2	m
51°35′ N, 173° E	8–VI	10	1	10	—	—	7_3	m
52°02′ N, 171°35′ E	5–VI	53	1	2	—	—	6_3	m
59°18′ N, 170°38′ E	31–VII	95	1	1	—	—	5_3	i
47°30′ N, 169°41′ E	26–V	55	—	—	2	4	$5_2,6_2$	m
50°10′ N, 168°15′ E	16–V	54	1	2	—	—	5_2	m
50°41′ N, 167°14′ E	15–VI	51	—	—	3	6	$5_2,6_2?(1)$	m
58° N, 166° E	22, 25, 26–VI	18	—	—	1	6	6_2	m
55° N, 164° E	29–VII	50	—	—	2	4	$4_2(2)$	i
55° N, 164° E	7–VIII	50	—	—	1	2	4_2	i
51°58′ N, 159°51′ E	26–VII	55	—	—	2	4	5_2	m
51°08′ N, 153°45′ E	15–VI	25	—	—	1	4	6_2	m
50°49′ N, 153°07′ E	5–VII	25	—	—	1	4	6_2	m

a—Numbers in parentheses following the age indicate the number of fish of that age. Where there is no number following an age it signifies that only one fish of that age was encountered. Sockeyes for which the age could not be determined, either because of poor scales, are indicated by? Where the freshwater zone of a scale could not be interpreted the ocean growth is indicated by the number of years followed by oc.

b—Fish which would have matured in the year they were caught are indicated by "m" and those that would have remained in the ocean at least one more year, i.e. immatures, are indicated by "i".

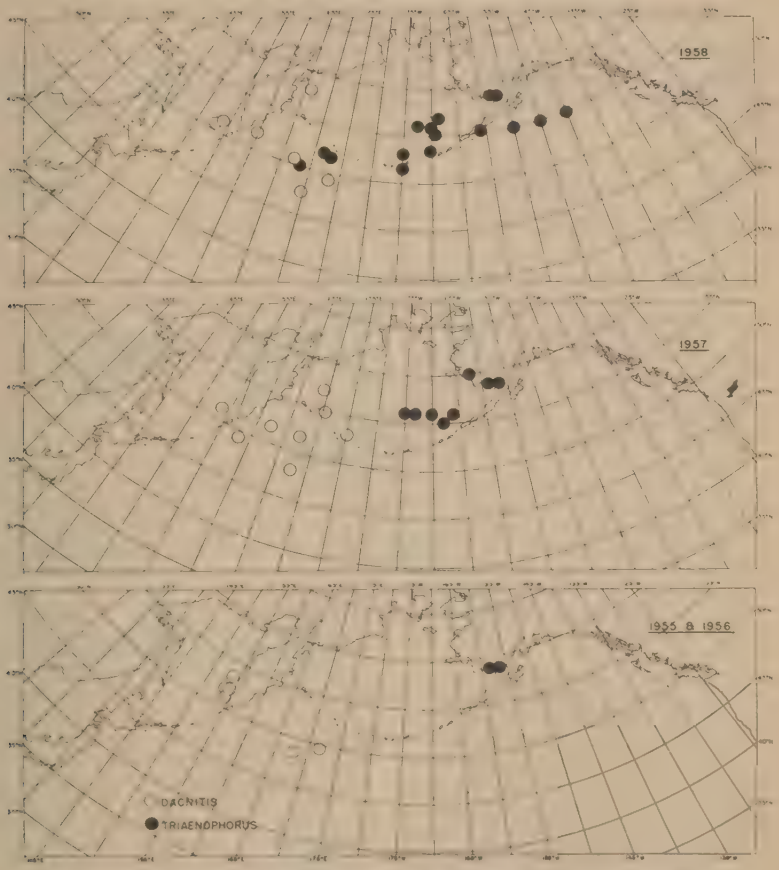


Figure 4. Distribution of maturing sockeye salmon infected with *Triaenophorus* or *Dacnitis* in the years 1955 to 1958.

or *Dacnitis* in 1958. *Triaenophorus*-infected maturing sockeye were found as far west as 168 15'E and eastward into the Gulf of Alaska to 145 W. *Dacnitis*-infected maturing sockeye were found from the Sea of Okhotsk eastward to 173 19'E.

The maturing sockeye infected with *Dacnitis* were caught from mid-May to the end of July, the earliest-caught fish generally having been taken farther to the east. The maturing sockeye infected with *Triaenophorus* (not including those taken in or near the estuaries of the home streams) were, with three exceptions, caught in May or June (see Table X). The three exceptions were: (1) one fish taken on July 10 in the commercial fishery operating in the Shumagin Islands area, Alaska, (2) one fish taken on July 2 at 53°01'N, 175°W and (3) one fish taken on August 5 at 51°30'N, 175°W. The occurrence of a maturing western Alaska sockeye in mid-ocean as late as August 5 seems very unusual, because it is generally believed that the sockeye returning to spawn in western Alaska have entered

Table XI. Incidence of *Triaenophorus* in adult sockeye runs to Bristol Bay in 1958, based on samples from each river.

River	Size of "run" (catch+escapement)	Sample size	% infected with *Triaenophorus*	Estimated no. infected in total "run"
Nushagak (Wood)	2,000,000	25	56	1,120,000
Kvichak	1,200,000	25	0	0
Naknek	575,000	52	2	11,500
Egegik	750,000	25	0	0
Ugashik	700,000	25	0	0
Totals	5,225,000			1,131,500

Estimated incidence of *Triaenophorus* in total Bristol Bay run – 21.7%

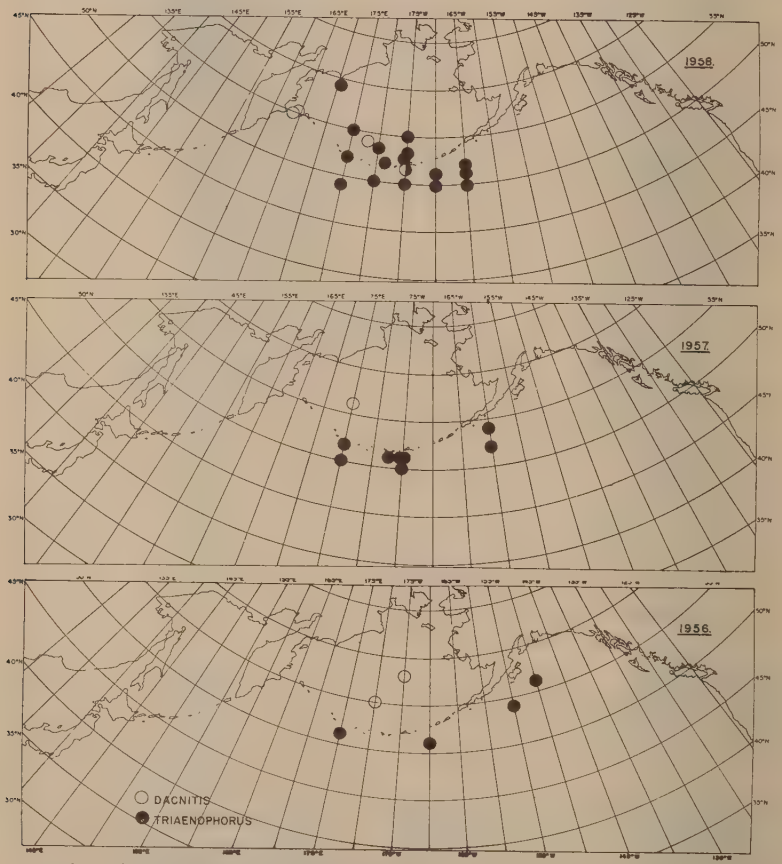


Figure 5. Distribution of immature sockeye salmon infected with *Triaenophorus* or *Dacnitis* in the years 1956 to 1958.

Table XII. Incidence of *Triaenophorus* in adult sockeye runs to Bristol Bay in 1957, based on samples from each river

River	Size of "run" (catch+escapement)	Sample size	% infected with *Triaenophorus*	Estimated no. infected in total "run"
Nushagak (Wood)	1,000,000	25	20	200,000
Kvichak	6,500,000	20	0	0
Naknek	1,500,000	25	12	180,000
Egegik	1,150,000	20	0	0
Ugashik	575,000	20	0	0
Totals	10,725,000			380,000

Estimated incidence of *Triaenophorus* in total Bristol Bay run = 3.5%

the rivers by the end of July or early August, but on the basis of gonad weight this sockeye definitely seems to be maturing. The fish was a female, age 4_2, with a body weight of 2,140 grams and a gonad weight of 88 grams. All other sockeye taken in the latter half of July or throughout the month of August, not only in 1958 but in other years as well, which were identified as being of western Alaskan origin because of the presence of *Triaenophorus*, were immatures. It appears that most of the maturing western Alaska sockeye leave the open ocean by the end of June, but some may remain there until early August.

The majority of maturing *Triaenophorus*-infected sockeye captured west of their area of origin (western Alaska) were from the Bering Sea between 175°W and 169°W, and were taken almost exclusively in the second half of June. The three *Triaenophorus*-infected sockeye from the area bounded by 168°E and 173°E Longitude and 50°N and 52°N Latitude were captured in May or early June. The apparent absence of *Triaenophorus*-infected sockeye between 173°E and 175°W may well be due to the fact that few samples taken from this area in May or June were examined. In the Gulf of Alaska the maturing *Triaenophorus*-infected sockeye were all taken in the first half of June.

Some features of the incidence of *Triaenophorus* in maturing sockeye were extremely interesting. On the basis of the small samples examined from the spawning runs to the rivers of Bristol Bay an overall incidence of *Triaenophorus* in all Bristol Bay sockeye (catch plus escapement) was calculated to be almost 22% (see Table XI). In the samples examined from 55°04′N, 169°24′W (79 sockeye), 53°30′N, 170°02′W (54 sockeye), 55°58′N, 170°02′W (23 sockeye) in the Bering Sea, and from 55°N, 155°W (72 sockeye) in the Gulf of Alaska, the incidence of *Triaenophorus* was either 22% or 24% (see Table X), which is identical or almost identical with the calculated overall percent incidence for Bristol Bay. This suggests that the samples from these high-seas localities consisted wholly or largely of a mixture of Bristol Bay sockeye, the various stocks being present in proportion to the size of their respective spawning runs.

In the Gulf of Alaska at 55°N, 150°W and at 55°N, 145°W the incidence of *Triaenophorus*-infected sockeye decreased to 9% and 4% respectively, and at 55°N, 135°W none were found, indicating a progressive decrease towards the east in the number of Bristol Bay sockeye in the samples. In the samples from 58°N, 145°W, 50°N, 145°W, and 50°N, 155°W *Triaenophorus* was not found.

Only one good sample was obtained from 170° to 175°E and therefore no data are available on the trends of *Triaenophorus* infection of sockeye in this area. In the 50 sockeye from 56°N, 172°30′W, the incidence of *Triaenophorus* was 8%. This lower rate of *Triaenophorus* infection as compared to the samples taken at 169°W and 170°W could mean that the sample did not consist solely of Bristol Bay sockeye, or that the various Bristol Bay stocks were not represented in proportion to the size of their respective runs, or that a greater proportion of uninfected than infected sockeye were represented from the stocks in which *Triaenophorus* occurred.

In the samples from south of the Aleutians between 173°E and 168°E only 3 maturing sockeye out of approximately 300 examined were found infected with *Triaenophorus*, suggesting a much lower proportion of Bristol Bay sockeye than in the samples from near 169°W and 170°W in the Bering Sea. Since good samples were also available to the west of 168°E and *Triaenophorus* was not found, 168°E probably was close to the extreme western limit of Bristol Bay or western Alaska sock-

eye, at least for the period during which the samples were collected.

Distribution of immature sockeye. The upper chart in Figure 5 shows the distribution of immature sockeye infected with *Dacnitis* or *Triaenophorus*.

Few *Dacnitis*-infected immature sockeye were found in the samples. One was taken just south of the Aleutian Islands at 175°W on August 5 in a sample which also contained several sockeye infected with *Triaenophorus*. Another was taken in the Bering Sea at 54°N, 178°E on July 15 and three were taken off the east coast of Kamchatka at 55°N, 164°E on July 29 or August 7.

Immature sockeye infected with *Triaenophorus* were represented in many of the samples from 165°W to 175°E which were collected from June 30 to August 19. One was also encountered in a sample of 95 fish from 59°18′N, 170°38′E taken on July 31, 1959. Most of the immature *Triaenophorus*-infected sockeye were taken south of the Aleutians, but some were also found in samples from north of the Aleutians to 55°N between 175°W and 175°E.

B. **Comparison of high-seas distribution of sockeye in 1958 with earlier years (1955–1957).** Figure 4 shows the distribution of maturing sockeye infected with *Dacnitis* or *Triaenophorus* for the years 1955 to 1958, inclusive.

One feature in the distribution of *Dacnitis*-infected maturing sockeye which is more or less similar from year to year is the most easterly point of capture, which is between 172°E and 175°E Longitude. It is impossible to determine if there was any change in the distribution of *Dacnitis*-infected sockeye from year to year because of their low incidence (never more than 11 in any one year and only 1.6% to 4% of all maturing sockeye taken from 175°E to the Sea of Okhotsk) and because the sampling locations, dates of sampling and the number of samples were not the same from year to year. For example, in the Bering Sea region in 1957 *Dacnitis* was found at 54°51′N, 170°24′E, and 57°02′N, 169°11′E. Samples from these locations were not examined in other years.

For between-year comparisons of the distribution of maturing *Triaenophorus*-infected sockeye, only 1957 and 1958 can be considered satisfactorily because of the lack or scarcity of samples in 1955 and 1956 from east of 175°E during the months of May and June.

In 1957 the qualitative distribution of *Triaenophorus*-infected maturing sockeye in the Bering Sea from 175°W and eastward was similar to that in 1958, but the incidence in this area was considerably less. Of the 263 fish examined from 166°W to 175°W, 3.4% were infected with *Triaenophorus* (incidence varying from 0% to 12% in individual samples) which is close to the calculated *Triaenophorus* incidence of 3.5% for all Bristol Bay runs combined, in 1957 (see Table XII). The much lower overall rate of *Triaenophorus* infection in the Bristol Bay stocks in 1957 as compared to 1958 is therefore reflected in the incidence in Bering Sea samples. This lower incidence was due to two factors: (1) The Wood River (Nushagak River system), which provides the majority of *Triaenophorus*-infected sockeye, comprised only about 10% of the 1957 Bristol Bay run, compared to about 40% in 1958, and (2) the incidence of *Triaenophorus* in the 1957 Wood River run was apparently lower than in 1958.

The samples from the Gulf of Alaska in 1957 were taken later in the season than those from comparable locations in 1958 (see 1958 Annual Report). If maturing Bristol Bay sockeye had been present in the Gulf of Alaska in 1957 they probably would have left the area on their homeward migration prior to commencement of the sampling. Therefore the absence of maturing sockeye infected with *Triaenophorus* from the Gulf of Alaska samples in 1957 may not indicate a real difference between 1957 and 1958.

Also, the failure to record any *Triaenophorus*-infected maturing sockeye in 1957 as far west as in 1958 cannot be interpreted as indicating that western Alaskan sockeye extended farther to the west in the latter year. In 1958, when the overall incidence of *Triaenophorus* in Bristol Bay sockeye was considerably higher (about 6 times) than in 1957, only 1% of the 300 sockeye examined in samples collected in May or early June between 168°E and 173°E were infected. This is about 1/20 of the overall incidence for Bristol Bay for that year. If the same relative representation of infected sockeye had prevailed in this area in 1957, then only about 0.17% would have been expected to be infected with *Triaenophorus*. Less than 100 maturing sockeye, taken in May or early June, from 175°E to 168°E were examined and the absence of *Triaenophorus* from these samples cannot lead to any conclusions regarding the presence or absence of Bristol Bay sockeye in this area in 1957.

Figure 5 shows the distribution of immature

sockeye infected with *Dacnitis* or *Triaenophorus* in the samples examined in the years 1956 to 1958.

Since few *Dacnitis*-infected immature sockeye have been observed in the samples there are insufficient data for a comparison between years. The most easterly records have been at 175°W in 1956 and 1958.

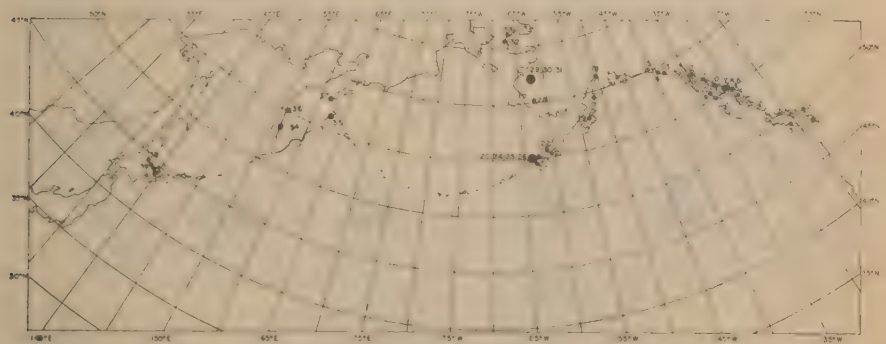
A constant feature of the distribution of immature sockeye infected with *Triaenophorus* is their occurrence south of the Aleutians to 175°E. The increased number of these immature sockeye observed from 1956 to 1958 is attributable in part to the increase in numbers of fish examined, but there was also an increase in the incidence of *Triaenophorus* in the immatures examined from samples taken from 175°E to 160°W. In 1956 1.5% of about 200 fish were infected, in 1957 3.3% of about 400 fish and in 1958 5% of about 600 fish.

Whether or not the occurrence of immature sockeye infected with *Triaenophorus* in the Bering Sea in 1958 indicates a real difference from other years cannot be determined because of differences in the number of fish examined and their localities and dates of capture.

3. STUDY OF CHUM SALMON SCALES TO DISTINGUISH STOCKS

In 1959, Canadian studies of chum salmon scales to distinguish stocks involved examination of scales collected in 1957 from coastal areas and spawning grounds throughout the North Pacific area. Scales from 3,576 chums from 33 inlets or river systems in North America and from 8 Asian river systems in Siberia and Japan were examined. The sampling areas and numbers of specimens examined from each are shown in Figure 6.

Age composition (Table XIII). In 1957,



Map No.	Location	No. in sample	Map No.	Location	No. in sample	Map No.	Location	No. in sample
1	Puget Sound	52	16	Cordova, Prince William Sound	58	30	Yukon River, Alakanuk	40
2	Fraser River	161						
3	W. Coast Vanc. Isl.	100	17	Seldovia, Rocky Bay	101	31	Yukon River, Far North	244
4	Skeena River	146	18	Cook Inlet, Kalgen Island	102	32	Teller, Pt. Clarence	87
5	Nass River	114						
6	Portland Canal	44	19	Uyak Bay	71	33	Kotzebue Sound	38
7	Boca de Quadra	12	20	Larsen Bay	150	34	Bolshaya River	49
8	Boca de Quadra, Anan Bay	7	21	Shumigan Island	249	35	Kamchatka River	49
			22	Pavlof Bay	196	36	Icha River	47
9	Noyes Island	11	23	Cold Bay	267	37	Uka River	25
10	Anan Bay	47	24	False Pass	51	38	Yubetsu River	33
11	Farragut Bay	15	25	Unimak Island	7	39	Tokoro River	59
12	Seymour Canal	72	26	Alitak	167	40	Abashiri River	162
13	Pt. Baker	14	27	King Cove	191	41	Shari River	92
14	Yakutat, East River	43	28	Kuskokwim River	74			
15	Taku River	46	29	Yukon River, Old Andreafsky	341			

Figure 6. Sampling locations for chum salmon utilized for 1957 scale studies.

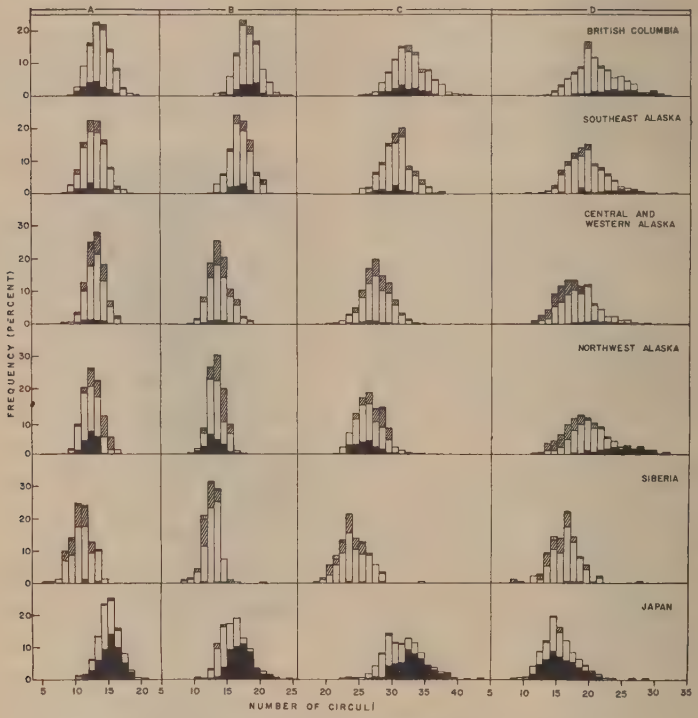


Figure 7. Distribution of circulus counts for scales of chum salmon from different areas of the North Pacific in 1957.
Column A—Circuli in first half of first-year band.
Column B—Circuli in last half of first-year band.
Column C—Total first-year circuli.
Column D—Total second-year circuli.
Black bars indicate frequencies for 3_1 fish, white for 4_1's and cross-hatched for 5_1's.

Table XIII. Percent age composition of chum salmon sampled in 1957 from 6 general areas.

Area	Age 3_1	4_1	5_1	6_1	7_1
British Columbia	18.2	75.7	6.1	—	—
Southeast Alaska	10.6	75.7	13.7	—	—
Central and Western Alaska	6.2	68.1	25.6	.1	—
Northwest Alaska	19.3	55.7	24.2	.8	—
Siberia	1.7	71.8	23.6	2.3	.6
Japan	51.7	45.4	2.9	—	—

chum salmon from the inshore waters of North America were again predominantly 4-year-olds, but the proportion of 3- and 5-year-olds was somewhat higher than in 1956. Siberian chums were also mainly 4-year-olds (70%). This is in contrast with data for 1956 when samples of chums taken off the Siberian coast contained roughly equal proportions of 4- and 5-year-old fish as well as a substantial number of 3-year-olds. Chums sampled in Japanese streams in 1957 were also mainly 4-year-olds but 3-year-olds were also well represented.

Circulus counts (Fig. 7). The 1956 data indi-

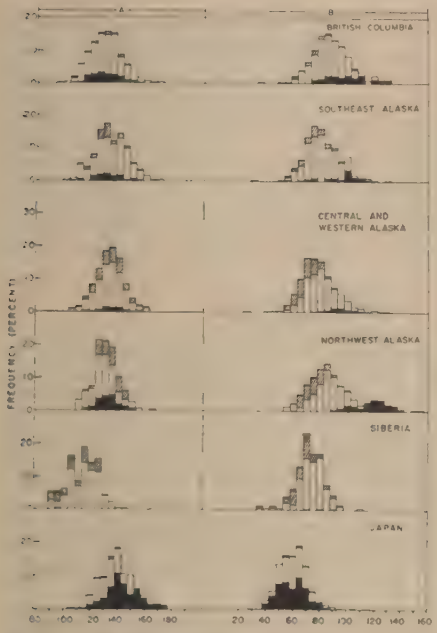


Figure 8. Widths of first- and second-year bands of scales of chum salmon from different areas of the North Pacific in 1957.

Column A—First-year annulus widths
Column B—Second-year annulus widths

cated a geographical cline in the number of **circuli in the first-year band,** fish from the southern extremes of the range tending to have more circuli than those from the more northern areas. The 1957 data show essentially the same trend. As seen in Figure 7, British Columbia (including State of Washington) and southeast Alaska chums had many closely packed circuli, more or less evenly distributed throughout the first-year band. Chums from the northwest part of North America (central, western and northwest Alaska) had fewer circuli, especially in the last half of the first-year band. Chums from Siberia had the fewest circuli of all. Japanese chums again exhibited a higher first-year circulus count, quite similar in magnitude to chums from British Columbia.

There was also a cline in the number of **circuli in the second-year band,** with the number of second-year circuli decreasing from East to West. Chums from British Columbia had the highest second-year circuli counts, whereas Japanese chums had the lowest (see Fig. 7).

Circulus counts for the first year for each area were quite similar for all age groups. Second-year counts, however, tended to vary with age, with the younger fish tending to have higher counts than the older ones (this tendency was evident for all stocks except the Japanese ones).

Annulus measurements (Fig. 8). Differences in annulus measurements between stocks tended

Table XIV. Percentage of chums from 6 general areas having first-year annulus widths of 1.05 mm or less.

Area	Percentage
British Columbia	2.61
Southeast Alaska	1.19
Central and Western Alaska	1.09
Northwest Alaska	0
Siberia	34.70
Japan	.58

Table XV. Percentage of chums from 6 general areas having total first-year circulus counts of 30 and more.

Area	Percentage
British Columbia	92.85
Southeast Alaska	78.72
Central and Western Alaska	26.89
Northwest Alaska	12.67
Siberia	.59
Japan	84.39

Table XVI. Proportion of chums from 6 general areas having first-year circulus counts of 30 and more and a difference of 0.45 mm or less between first- and second-year annulus widths.

Area	Percentage of sample
British Columbia	57.6
Southeast Alaska	27.2
Central and Western Alaska	5.7
Northwest Alaska	1.9
Siberia	0
Japan	0.6

to parallel the observed differences in circulus counts. First-year annuli in the southern-most extremes of the chum salmon's range (i.e. British Columbia and Japan) tended to be larger than those in the northern areas. For second year annuli, however, there was an East-West cline in annulus widths; scales from British Columbia chums had the largest second-year widths, while those from Japanese chums had the smallest.

Some distinguishing characteristics of Asian and North American chums. As seen in Figure 8, Siberian chum salmon tended to have narrower first-year annuli than did chums from other areas. Fish with first-year annuli measuring 1.05 mm and less composed 35% of the samples collected from Siberian streams, whereas they formed less than 3% of the fish sampled in other areas. The percentage of fish having first-year annulus measurements of 1.05 mm and less for each of the six general areas of study are listed in Table XIV.

As noted in Figure 7, fish having first-year circulus counts of 30 and more formed the major proportion of samples from British Columbian, southeast Alaskan and Japanese coastal areas. Only one fish of the 170 sampled from Siberia exhibited a circulus count as high as 30 (Table XV). It can also be seen from Figure 7 that although fish from Japan had high first-year circulus counts similar to those for British Columbian and southeast Alaskan fish, they had considerably lower second-year circulus counts and annulus widths. On the basis of these observations, for fish from each of the six general areas, a comparison was made of the difference between the first-year and second-year annulus widths for fish with 30 and more circuli in the first year. It was found (Table XVI) that chums having 30 and more first-year circuli and differences between the widths of the first and second annuli of 0.45 mm and less formed over half of the fish sampled in British Columbia, approximately one-quarter of the southeast Alaskan fish, less than 10% of the fish sampled in the more northern North American streams and virtually none of the fish from the Asian samples (only 2 out of 516 fish examined).

4. OCEANOGRAPHY

A. **North Pacific surveys.** Two oceanographic surveys co-ordinated with the research program of the Commission were accomplished this year. The first, a winter survey, was carried out between January 20 and February 15 aboard C.N.A.V. *Oshawa* north of Latitude 47°N and east of Longitude 157°W. The second, a summer survey, was carried out during the period August 4 to September 1. Two ships, C.N.A.V. *Oshawa* and C.N.A.V. *Whitethroat*, were used and they covered the area north of Latitude 38°N and east of Longitude 160°W to the North American coast. Observations at Ocean Weather Station "P" (Latitude 50°N, Longitude 145°W) were continued, with sections at six-week intervals between that point and the coast.

January—February, 1959. The observed temperature distribution at 10 metres depth (a more

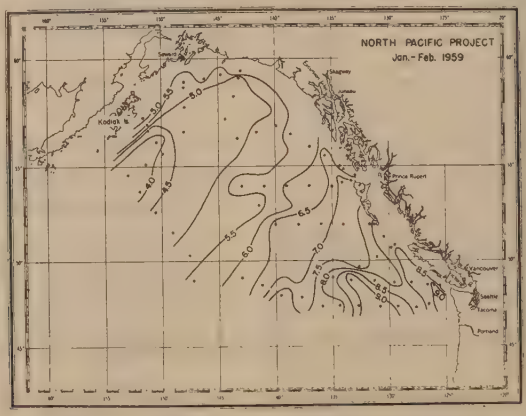


Figure 9. Temperature (°C.) at 10 meters, Jan.–Feb. 1959.

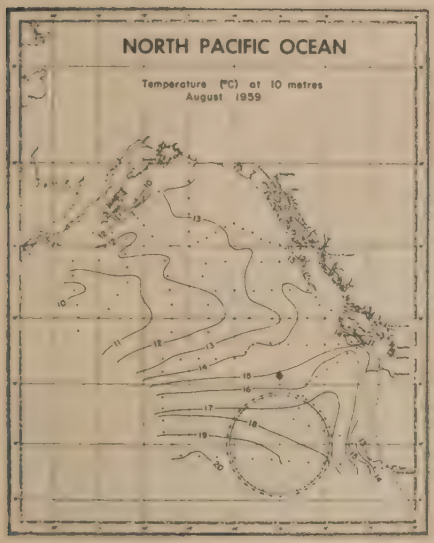


Figure 10. Temperature (°C.) at 10 meters, August 1959.

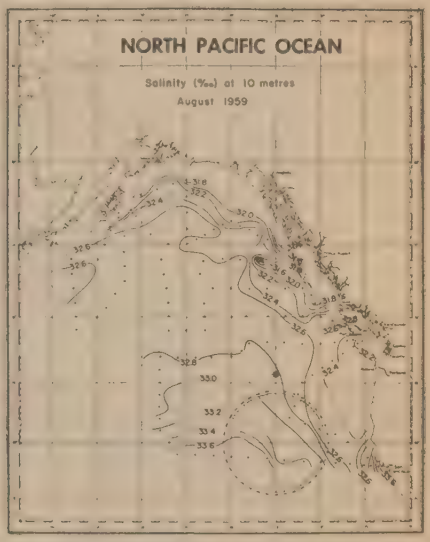


Figure 11. Salinity (‰) at 10 meters, August 1959.

Figure 12. Temperature (°C.) on the surface, August 1959.

realistic level than the surface) is shown in Figure 9. Temperatures in the Alaska Gyral and mid-ocean region were practically the same as in March—April, 1958. However, around the coast the water was about one degree (°C) colder than in the previous winter, but still warmer than the average. These data, however, do not represent the winter minimum, which occurred in late March, as shown by the data from Ocean Station " P " (Lat. 50°N, Long. 145°W).

The salinity distribution at 10 metres depth was similar to that observed during the previous winter. The high salinity centre (<32.8‰) south of Kodiak Island, which was absent during the previous summer, was again present. As usual, there was a band of low salinity water close along shore, all around the coast. At 100 metres depth high salinity (<33.4‰) was present off Vancouver Island and southeast of Kodiak Island. The cells of low salinity water, observed during the previous summer, had dissipated at the time of this survey.

August, 1959. The temperature distribution at 10 metres depth (Fig. 10) was similar to that observed during August, 1956. Between Ocean Station " P " and the coast the water was considerably cooler (3°C) than during August, 1958 and some-

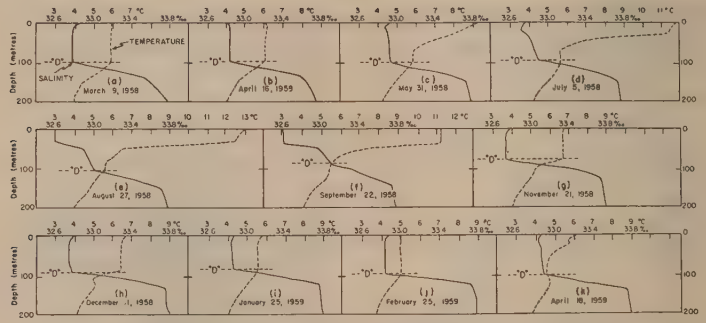


Figure 13. Salinity (—) and temperature (- -) structure at Ocean Station " P " (lat. 50°N, long. 145°W), March 1958–April 1959.

what cooler than during August, 1957. Similar conditions existed at the surface at Ocean Station " P " during this period. Immediately off the coast of Vancouver Island temperatures were relatively cold, being similar to those observed during the previous summer.

The salinity distribution (Fig. 11), unlike the temperature distribution at 10 metres depth, was more similar to that observed during the summers 1957 and 1958 than that observed during the summer 1956. However, the 32.6‰ isohaline did not extend as far offshore as observed during the two previous periods.

The salinity and temperature distributions suggest " upwelling " off the coast of Vancouver Island and the California coast.

The warm water intrusion. The distribution of temperature on the surface σ_t=26.60 (density 1.02660) has been used in earlier reports to show the intrusion of warm water below the level of seasonal influence since 1956 (see Fig. 20, Annual Report for 1958). The distribution of temperature on this surface for August, 1959, is shown in Figure 12. This temperature distribution was very similar to that observed during August, 1956, particularly off the British Columbia coast.

B. **Depth of seasonal influence in the sub-Arctic Pacific Ocean.** Previous reports have shown that the salinity structure, with minor variations, is a permanent feature of the eastern sub-Arctic and this structure could be accurately delineated by plotting salinity as a function of the logarithm of the depth. Such a plot leads to a definition of three distinct zones; an upper zone of relatively homogeneous low salinity water, a transition zone characterized by a marked halocline, and a lower zone in which the salinity increases gradually with depth. These are separated by two marked discontinuities at the upper and lower limit of the halocline. They have been designated " D " and " L " respectively.

A study of the upper zone now has been completed on all available data for the sub-Arctic region of the North Pacific Ocean for the periods 1956 through 1959. As a result it can now be shown that an upper zone, and a limit " D ", can be defined throughout the sub-Arctic region. The lower limit " L ", however, is not an invariable feature of this region. In areas of mixing such as the vicinity of the Aleutian Islands and in some areas of the Bering Sea the marked halocline deepens and the lower limit " L " becomes indefinite. It has also been shown that in the western sub-Arctic, when the lower limit of the halocline can be defined, the salinity at " L " approximates 33.8‰, very near the values that have been reported previously for the eastern sub-Arctic.

It is now apparent from an examination of data at Ocean Station " P " (Fig. 13) that the upper zone is established during the period of cooling, and although it undergoes changes, it can be defined throughout the period of heating, April—September, and is again re-established during the winter period when surface cooling and mixing by either wind action or convection, or both, produces isohaline and isothermal structures to " D ". These processes do not extend below " D ".

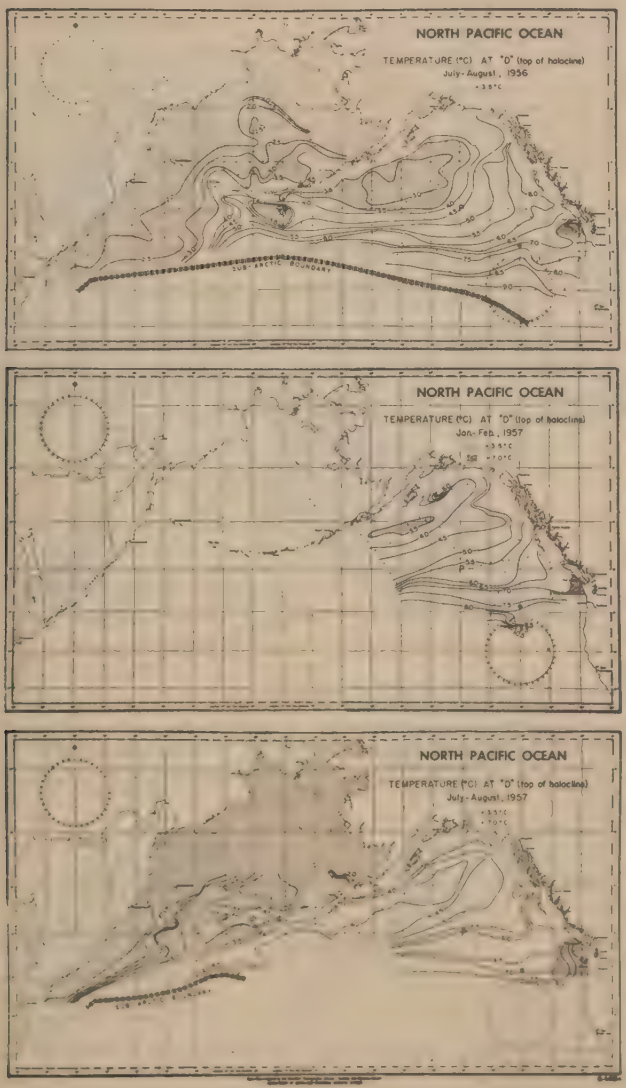

NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "0" (top of halocline)
July - August, 1956
SUB-ARCTIC BOUNDARY
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "0" (top of halocline)
Jan.-Feb., 1957
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "0" (top of halocline)
July - August, 1957

Figure 14.

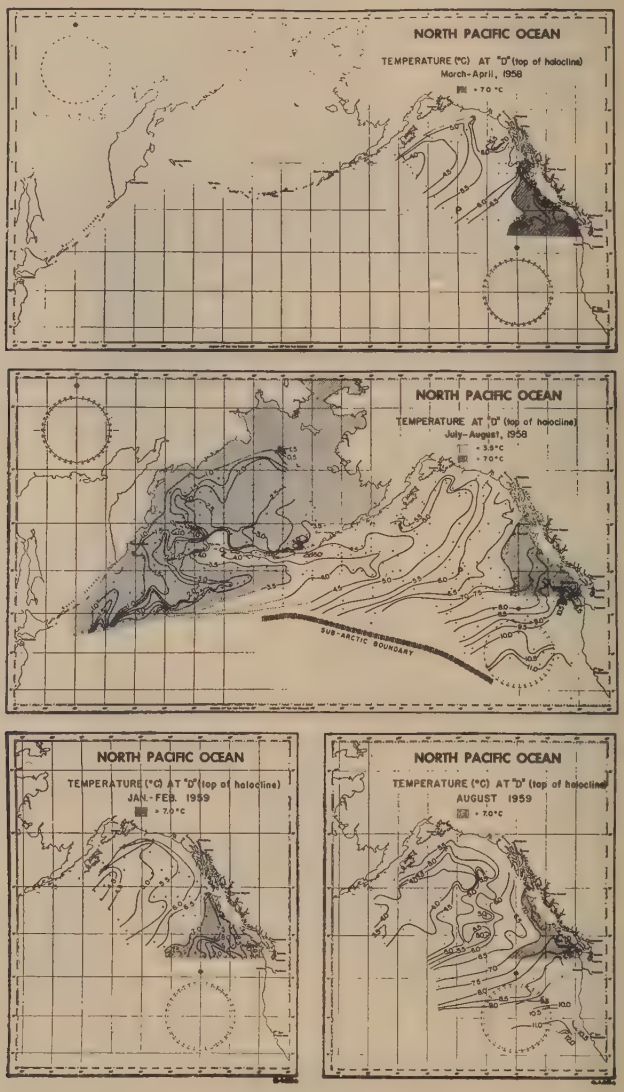

NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
March-April, 1958
NORTH PACIFIC OCEAN
TEMPERATURE AT "D" (top of halocline)
July-August, 1958
SUB-ARCTIC BOUNDARY
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
JAN.-FEB. 1959
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
AUGUST 1959

Figure 15.

Changes that occur in the salinity structure during the period of heating can be related to the development of the marked thermocline within the upper zone and the " entrainment " processes.

There is evidence to suggest that, at least in the eastern sub-Arctic Pacific Ocean, the establishment of the isohaline and isothermal upper zone is primarily due to cooling and mixing by wind action. It is further proposed that the extremes of temperature and salinity established in the upper zone during the winter can be recognized in the summer data at depth " D ". The absolute values are altered slightly by " entrainment " (salinity 0.2‰; temperature 0.2–0.5°C).

Thus it is possible to recognize, within small limits, the winter distribution of temperature in the whole of the upper zone from the summer data at " D ". This can be seen from an examination of the winter and the following summer distributions in the eastern sub-Arctic (Fig. 14 and 15) for the years 1957 and 1958. It has been proposed that these conclusions are applicable for the whole of the sub-Arctic region for these periods.

It is noted from a casual inspection, that the summer distribution of temperature at " D " is not as representative of the actual winter distribution for 1959 as was the case for the previous periods. The apparent discrepancy between these two periods can be explained. First, there was fairly marked cooling after the completion of the winter survey (January, 1959). Thus the minimum of winter conditions was not observed. This was not the case in the other winter surveys. Secondly, it is suggested that there has been a change in the general trend of warming which has occurred from 1955 through 1958. It appears that the circulation was returning to that observed during 1956. These two factors can account for the differences observed.

These figures reflect the marked variations that have occurred during these periods. It is interesting to note that warming in the eastern sub-Arctic was accompanied by cooling in the western sub-Arctic.

MARINE STEROLS

IX. BIOSYNTHESIS OF 24-METHYLENECHOLESTEROL IN CLAMS[1]

U. H. M. Fagerlund and D. R. Idler

Abstract

Clams (*Saxidomus giganteus*) are able to transform injected cholesterol-4-C^{14} into radioactive 24-methylenecholesterol in vivo. The highest rate of conversion was observed when the radioactive sterol was injected into the digestive gland. The findings suggest a possible pathway in the biosynthesis of C_{28}-sterols and throw some light on the question of the origin of C_{28}-sterols in lamellibranchs.

Introduction

Previous experiments (1) conducted in this laboratory have shown that two members of the phylum Mollusca, a mussel (*Mytilus californianus*) and a clam (*Saxidomus giganteus*), are able to synthesize a sterol from injected acetate-2-C^{14}. The chromatographic properties of the sterol are consistent with those of a monounsaturated Δ^5-sterol. Only a trace of radioactivity was found in the chromatographic zone containing 24-methylenecholesterol. This observation is significant in view of the fact that 24-methylenecholesterol constitutes more than 50% of the total sterols in clams. It would seem that either the turnover of 24-methylenecholesterol is very slow or this sterol is of exogenous origin. The present investigation reveals that injected cholesterol-4-C^{14} is converted to radioactive 24-methylenecholesterol in the clam. Thus it appears that at least part of the 24-methylenecholesterol found in clams is supplied by biosynthesis within the animal.

Experimental

Radioactivity was determined with a gas-flow counter equipped with a "micromil" window and specially shielded for low background counting.* The background count obtained with this instrument was of the order of 2 c.p.m. Melting points were determined either with a conventional melting point apparatus or on a Kofler block.

Procedure for Chromatography of Sterols and Steryl Acetates on Paper

A method for the chromatographic separation of sterols and steryl acetates on paper has been described by deZotti *et al.* (2). This method was adopted with slight modifications. Commercial kerosene was distilled and the fraction boiling at 220–240° collected. Whatman No. 1 chromatographic filter paper was impregnated with this fraction by dipping it into a 12% solution of kerosene in dichloromethane. Dichloromethane was evaporated by letting the paper hang in air a few minutes. In this way impregnation of approximately 150 mg dm^2

[1]Manuscript received January 5, 1961.
Contribution from the Fisheries Research Board of Canada, Technological Station, Chemistry Section, Vancouver 8, B.C.

*Supplied by Nuclear-Chicago Corp., Chicago 10, Ill., U.S.A.

was obtained. The material to be chromatographed was then applied as quickly as possible to a line 8 cm from one end of the paper strip. Sterols were run as 20 μg spots and steryl acetates as 50 μg spots. Larger amounts were applied to the origin as a strip the length of which varied with the amount of compound.

Chromatographic jars were equilibrated with the mobile phase for 24 hours previous to chromatography. Sterol chromatograms were developed with 85% aqueous pyridine by ascending chromatography at 20° for 24–40 hours. Steryl acetate chromatograms were developed with 98% aqueous acetic acid by ascending chromatography at 25° for 24–48 hours.

Eighty-six hours was required for the solvent front to reach the top of a 55-cm paper strip. However, after this time, the spots were often diffuse. Both sterols and steryl acetates were sometimes developed by descending chromatography. The time of development was then much shorter.

After evaporation of the mobile phase the chromatograms were sprayed with a saturated solution of $SbCl_3$ in a 1:1 mixture of acetic acid and chloroform. They were heated in a drying cabinet at 80–90° for 5–10 minutes. Strips were located by cutting off and spraying a small portion of the chromatogram containing some of the material on each side of the strip. Standards were run on each side of a strip.

When chromatographed as described, ketosteryl acetates run with a speed more than twice that of the corresponding monohydroxysteryl acetates. Table I shows the separation obtained with certain pairs of steryl acetates.

TABLE I

Approximate distance between centers of spots after chromatography of steryl acetates on paper

	24 hours	48 hours
24-Ketocholesterol—25-ketocholesterol	2.3 cm	3.1 cm
24-Methylenecholesterol—25-dehydrocholesterol	1.5 cm	1.5–2.1 cm
Sitosterol—cholesterol	1.3 cm	

The acetate with the smaller molecular weight is faster-running. Cholesteryl acetate is slower than either 24-methylenecholesteryl acetate or 25-dehydrocholesteryl acetate. A somewhat better separation was obtained with free sterols.

20-Dehydrocholesteryl acetate and its ozonolysis product, 20-keto-21-norcholesteryl acetate, have nearly the same mobility as 24-methylenecholesterol acetate and 24-ketocholesteryl acetate, respectively, and a separation was not possible.

Experiment I

Injection of Cholesterol-4-C^{14} and Isolation of 24-Methylenecholesterol

Clams were kept during the month of July in an aquarium supplied with running, aerated sea water. Cholesterol-4-C^{14},* with a specific activity (S.A.)

*Supplied by Beta Laboratories Inc., Philadelphia, Pa., U.S.A.

of 4.05×10^6 c.p.m./mg, was dissolved in 0.2 ml of salmon oil, which was injected at random into two clams with a 22-gauge hypodermic needle forced in between the closed shells. No attempt was made to establish the amount of cholesterol-4-C^{14} actually received by the tissues of the clams, since losses which are difficult to determine quantitatively occur during the injection. One week after the injection, the soft parts of the animals were macerated in a Servall Omni-Mixer and extracted three times with hot acetone. The solution was concentrated to an oil (2.8×10^7 c.p.m.) which was saponified with potassium hydroxide. The non-saponifiable portion was extracted from the saponification mixture in the usual manner and was then chromatographed on neutral aluminum oxide "Woelm"† (activity grade II). The sterol fraction was eluted with a 1:1 mixture of ethanol and dichloromethane. The *p*-phenylazobenzoyl esters of the sterol mixture were prepared (3) and chromatographed on silicic acid – Celite 535 using petroleum ether-benzene (5.5:1) to develop the chromatogram (4). Two colored zones developed. The lower zone contained unchanged cholesterol-4-C^{14}. The upper zone, which, according to our previous experiments (5), contained 24-methylenecholesterol, was eluted. This zone was contaminated with some cholesteryl azoate of high specific activity probably caused by surface spreading (4) of the lower zone. In order to remove most of this material the upper zone azoate was chromatographed together with carrier cholesteryl azoate. The upper zone was again eluted and the same procedure was repeated once more. The 24-methylenecholesteryl azoate was finally crystallized from benzene – aqueous methanol to remove impurities originating from the adsorbent, and hydrolyzed. The free sterol weighed 57 mg and had a radioactivity of 44,550 c.p.m. or a S.A. of 782 c.p.m./mg. The major portion of the radioactivity found in the non-saponifiable matter was recovered from the combined cholesteryl azoate zones (2.3×10^7 c.p.m.).

Radioactive 24-methylenecholesterol (9.8 mg) obtained through hydrolysis of the upper zone azoate was mixed with 44.1 mg of authentic 24-methylenecholesterol previously isolated from clams and the mixture was acetylated. The acetate was crystallized 8 times from aqueous methanol. The S.A. diminished from 129 c.p.m./mg to 39.6 c.p.m./mg without reaching a constant value.

Supernatants from the above crystallizations were combined and the combined material crystallized twice from aqueous ethanol. The S.A. was then 66 c.p.m./mg. The material (1.11 mg) was chromatographed on paper as a 10-cm long strip for 24 hours. After the zone had been located in the usual manner it was eluted and rechromatographed for 66 hours. The S.A. of the twice chromatographed acetate was 30.3 c.p.m./mg.

Ozonolysis of Upper Zone Acetate and Isolation of 24-Ketocholesteryl Acetate

Radioactive 24-methylenecholesterol (15 mg) from the upper zone of the azoate chromatogram was combined with 76 mg of authentic 24-methylenecholesterol obtained from clams. The mixture was acetylated. The acetate

†Supplied by Mapharm Chemicals, P.O. Box 755, New Orleans, La., U.S.A.

was suspended in a 4:1 mixture of acetic anhydride and acetic acid and ozonized in an ice–salt bath. During the ozonolysis most of the acetate dissolved in the reaction mixture. Zinc dust and water were added to the solution and the ozonides decomposed by boiling for 20 minutes. After the solution had cooled an excess of water was added and steryl acetates were recovered by extraction with ether, washing with $NaHCO_3$, and evaporation of the ether. The acetate mixture was chromatographed on aluminum oxide of activity grade II. Unreacted steryl acetate was eluted with heptane–benzene (1:1). The ketonic material was eluted in two fractions with benzene–dichloromethane (1:1).

Fraction 1 had a radioactivity of 488 c.p.m. It was crystallized 4 times from aqueous methanol. A slight change in S.A. occurred in the first crystallization, but subsequent values remained constant, viz. 26.5 c.p.m./mg.

Fraction 2 of the ketonic material obtained by chromatography of the ozonolysis products had a radioactivity of 914 c.p.m., but the weight of the crude material left after evaporation of the solvent was only 3 mg. One half of this fraction was applied as a strip to a paper chromatogram and chromatographed for 23 hours. The major part of the $SbCl_3$ positive material had travelled the same distance as the 24-ketocholesteryl acetate standard, namely 17.7 cm from the origin. There was some material in the 25-ketonorcholesteryl acetate section and part of the substance had travelled ahead of 25-ketonorcholesteryl acetate. Four sections were cut out, eluted, and counted: section 1 (27.5 c.p.m., 2 cm wide) with the motility of 24-ketocholesteryl acetate, section 2 (96.6 c.p.m., 2 cm wide) with that of 25-ketonorcholesteryl acetate, section 3 (36.5 c.p.m., 2.3 cm wide) immediately ahead of the latter, and section 4 (12.0 c.p.m., 3.0 cm wide) immediately behind 24-ketocholesteryl acetate. The material from Sections 1 and 4, was rinsed off the planchets, combined, and rechromatographed as an 8 cm long strip for 24 hours. When the chromatogram was dried, and the various sections cut out and eluted, the 24-ketocholesteryl acetate section (2 cm wide) carried 11.6 c.p.m., the 25-ketonorcholesteryl acetate section (2 cm wide) carried 6.8 c.p.m., and the section immediately behind 24-ketocholesteryl acetate (2 cm wide) carried 4.8 c.p.m. The S.A. of 24-ketocholesteryl acetate from this chromatogram was 27.2 c.p.m./mg.

Separation of 20-Keto-21-norcholesteryl Acetate from 24-Ketocholesteryl Acetate by Crystallization

A mixture of 1.8 mg of 20-keto-21-norcholesteryl acetate (plates, m.p. 147°) and 4.35 mg of 24-ketocholesteryl acetate (needles, m.p. 129°) was crystallized from aqueous methanol. Melting points recorded were: I, 105–120°; II, 120–124°; III, 127°; V, 128°. The acetates of later crystallizations formed needles. The melting point was markedly depressed when the material was mixed with 20-keto-21-norcholesteryl acetate. A gas chromatographic analysis of the final product (V) revealed it to be 24-ketocholesteryl acetate, which contained less than 4% impurities.

The remaining half of fraction 2 from the column chromatography of the

ozonolysis products of radioactive 24-methylenecholesteryl acetate was mixed with 2.65 mg of 20-keto-21-norcholesteryl acetate. The mixture (m.p. 105–110°) was crystallized 8 times from aqueous methanol. The S.A. of the crystallized material (needles, m.p. 135–137°) was only 1.4 c.p.m./mg. Gas chromatographic analysis was made of a sample obtained by pooling the supernatants from the last three crystallizations and crystallizing the pooled material once. According to the analysis, this sample was 89% pure 20-keto-21-norcholesteryl acetate containing 8% of 24-ketocholesteryl acetate.

Experiment II

Injection of Cholesterol-4-C^{14} and Isolation of 24-Methylenecholesterol

Five clams, 2–3 years of age, taken in the month of February were kept in seawater (aerated, but not running) at 17°. Cholesterol-4-C^{14}* (S.A., 8.25×10^7 c.p.m./mg) was dissolved in a few drops of olive oil. The oil was injected into the digestive glands by inserting the needle between the closed shells close to the hinge. The animals apparently did not suffer any ill effects from the injections. Four clams (batch 1) were kept for 4–9 days in an aquarium, and one clam (batch 2) was kept for 11 days. The water in the aquarium was changed once, on the third day after the injection.

The procedure for extraction of the oil and for obtaining the non-saponifiable matter has been described in experiment I. There were 7.39×10^6 c.p.m. in the oil from batch 1. 24-Methylenecholesterol from this batch was obtained by chromatography of the non-saponifiable matter (6.83×10^6 c.p.m.) on paper without preliminary purification. Approximately 1.3 mg (4230 c.p.m.) was chromatographed in the 85% pyridine chromatographic system. The section containing 24-methylenecholesterol (259 c.p.m.) was cut out. The sterol was eluted and rechromatographed in the same system. 24-Methylenecholesterol obtained from this chromatogram weighed 0.142 mg (86.7 c.p.m.). The material was mixed with 1.265 mg of authentic 24-methylenecholesterol and crystallized twice from aqueous methanol. The S.A. dropped sharply in the first crystallization, but changed only slightly in the second. The diluted material was finally chromatographed on paper, and the compound obtained from the 24-methylenecholesterol area was crystallized once. The S.A. was slightly higher, 20.6 c.p.m./mg.

There were 2.36×10^6 c.p.m. in the oil fraction from batch 2. To isolate the sterols, the non-saponifiable portion (1.48×10^6 c.p.m.) was chromatographed on alumina of activity grade II as described in experiment I. The sterol fraction weighed 33.7 mg (1.29×10^7 c.p.m.).

This fraction was ozonized and resulting ketosteryl acetates were isolated as described above in experiment I. They were chromatographed on paper in the 98% HAc system. The 24-ketocholesteryl acetate section was cut out and the acetate eluted and rechromatographed in the same system. Thus 0.461 mg (1150 c.p.m./mg) of 24-ketocholesteryl acetate was obtained. The material

*Supplied by Merck & Co. Ltd., Montreal, Que.

was mixed with authentic 24-ketocholesteryl acetate (3.163 mg) and the mixture was crystallized repeatedly from aqueous methanol. After an initial sharp drop in the first crystallization, the S.A. remained constant at 38.5 c.p.m./mg. On the basis of this value, the S.A. of the original 24-methylenecholesterol was calculated to be 336 c.p.m./mg.

Results

Experiment I

Cholesterol-4-C^{14} was injected at random into clams, which were held in an aquarium for 7 days. After extraction of the clams, saponification of the oil, and chromatography of the non-saponifiable matter, 24-methylenecholesterol was isolated from the sterol mixture through chromatography of the azoyl esters. The free sterol was acetylated and mixed with carrier 24-methylenecholesteryl acetate and the mixture was crystallized several times. 24-Methylenecholesteryl acetate was separated from any 25-dehydrocholesteryl acetate present and from residual cholesteryl acetate by chromatographing the crystallization product twice on paper. The S.A. of 24-methylenecholesterol acetate was then 30.3 c.p.m./mg. The true S.A. of the original 24-methylenecholesterol before dilution was calculated on the basis of this value and was found to be 184 c.p.m./mg.

Another sample of 24-methylenecholesterol obtained from the azoyl ester chromatogram was mixed with carrier 24-methylenecholesterol; the mixture was acetylated, and ozonized. After splitting of the ozonides, crude 24-ketocholesteryl acetate was separated from unreacted material on alumina. A portion of the 24-ketocholesteryl acetate fraction (fraction 1) was crystallized to a constant S.A. of 26.5 c.p.m./mg. The S.A. of the original undiluted 24-methylenecholesterol calculated on the basis of this value would be 179 c.p.m./mg.

Another portion (fraction 2) of the ozonolysis product was purified by chromatography on paper. After being chromatographed twice, the 24-ketocholesteryl acetate had a S.A. of 27.2 c.p.m./mg or the same as obtained by crystallization. Fraction 2 originally had a much higher S.A. The major part of the radioactivity had the same mobility on the first paper chromatogram as 25-ketonorcholesteryl acetate. The extent of conversion of cholesterol-4-C^{14} to 24-methylenecholesterol was calculated on the basis of the average S.A. (26.9 c.p.m./mg) of 24-ketocholesteryl acetate. Taking into account a 24-methylenecholesterol content of approximately 80 mg in the two clams, the radioactivity in 24-methylenecholesterol was 0.052% of the total radioactivity extracted from the animals with acetone.

Experiment II

Cholesterol-4-C^{14} was injected into the digestive glands of five clams. The clams used in this experiment were younger than the ones in the previous experiment. Four clams (batch 1) were kept from 4 to 9 days. One clam (batch 2) was kept for 11 days before being extracted.

24-Methylenecholesterol was isolated from the non-saponifiable matter obtained from batch 1 by chromatography on paper. After the sterol had been chromatographed on paper once more it was mixed with carrier 24-methylenecholesterol and crystallized to a constant S.A. of 18.9 c.p.m./mg. The diluted material was chromatographed on paper and thereafter crystallized once. The S.A. was 20.6 c.p.m./mg. The S.A. of original, undiluted 24-methylenecholesterol calculated on the basis of this value was 204 c.p.m./mg. There was 87 mg of 24-methylenecholesterol present in the acetone extract of this batch as determined by chromatographic separation of the azoyl esters. The radioactivity carried by 24-methylenecholesterol represents 0.25% of the total radioactivity extracted from the animals with acetone.

The total sterol fraction from batch 2 was acetylated, ozonized, and the ozonides decomposed. Ketosteryl acetates were separated from unreacted material by chromatography of the ozonolysis products on alumina and were twice chromatographed on paper. 24-Ketocholesteryl acetate, thus obtained, was mixed with authentic 24-ketocholesteryl acetate and crystallized to a constant specific activity of 38.5 c.p.m./mg. The S.A. of original, undiluted 24-methylenecholesterol determined on the basis of this value was 336 c.p.m./mg. There was 34 mg of sterols in this batch. Assuming that the ratio of the amount of cholesterol to that of 24-methylenecholesterol is the same as in batch 1, the radioactivity carried by 24-methylenecholesterol from this batch represents 0.30% of the total radioactivity extracted from the animal with acetone.

The only known sterols which might possess chromatographic and solubility properties similar to those of 24-methylenecholesterol and which could be ozonized as acetates to steryl acetates with chromatographic and solubility properties similar to those of 24-ketocholesteryl acetate are 25-dehydrocholesterol, 20-dehydrocholesterol, and fucosterol (24-ethylidene cholesterol). Previous work (6) in this laboratory has shown that, when radioactive cholesterol is injected into clams, a radioactive C-25 unsaturated sterol, believed to be 25-dehydrocholesterol, accompanies 24-methylenecholesterol when the azoates are chromatographed. In the present investigation the high radioactive count in fraction 2, experiment I, from the alumina chromatogram of the ozonolysis products indicates the presence in the sterol fraction of 25-dehydrocholesterol carrying a higher S.A. than that of 24-methylenecholesterol. To exclude contamination assignable to this source, samples of radioactive 24-methylenecholesteryl acetate and its ozonolysis product have been chromatographed on paper in systems that separate these compounds from the corresponding 25-dehydrocholesterol derivatives.

The second possible contaminant, 20-dehydrocholesteryl acetate, did not separate effectively from 24-methylenecholesteryl acetate by the paper chromatographic method used in this investigation. This is the case also in regard to the corresponding ketosteryl acetates. However, it is possible to separate the ozonolysis product, 20-keto-21-norcholesteryl acetate, from 24-ketocholesteryl

acetate by crystallization. When a mixture of 1.8 mg of the former and 4.35 mg of the latter was crystallized 5 times from aqueous methanol, the latter was obtained reasonably free from 20-keto-21-norcholesteryl acetate as evidenced by melting point, mixed melting point, and gas chromatographic analysis. Since it was possible to crystallize the ozonolysis product of 24-methylenecholesteryl acetate to a constant S.A. it follows that the radioactivity is not attributable to 20-keto-21-norcholesteryl acetate.

Additional evidence for the absence of any appreciable amount of radioactive 20-keto-21-norcholesteryl acetate in the radioactive 24-ketocholesteryl acetate was obtained by crystallization of a small amount of radioactive 24-ketocholesteryl acetate (fraction 2) mixed with a larger amount of 20-keto-21-norcholesteryl acetate. It is possible to separate the two compounds by crystallization even when they are present in this proportion as evidenced by melting point and gas chromatographic analysis. If the radioactivity attributed to 24-ketocholesteryl acetate had, in fact, been caused by the presence of a minor amount of radioactive 20-keto-21-norcholesteryl acetate, then the sample should have retained this activity. From the results of a chromatographic analysis of fraction 2, it was estimated that the material mixed with 20-keto-21-norcholesteryl acetate contained more than 1 mg of 24-ketocholesteryl acetate. If the radioactivity in this sample (>27.2 c.p.m.) was attributable to 20-keto-21-norcholesteryl acetate, then the final crystallization product (20-keto-21-norcholesteryl acetate) when free from 24-ketocholesteryl acetate would have had a S.A. greater than 27.2 c.p.m./2.65 mg or greater than 10.3 c.p.m./mg. However, the S.A. diminished to a value of 1.4 c.p.m./mg through eight crystallizations.

The third possible contaminant of the radioactive 24-methylenecholesterol, fucosterol, is ruled out because of the difference in chromatographic properties. In the 85% acetic acid system, fucosteryl acetate is well separated from the faster-running 24-methylenecholesteryl acetate.

Discussion

Carbon-28 in the molecule of sterols and triterpenes is known to be derived from one-carbon donors, such as formate, serine, and methionine (7, 8, 9). However, little information is available relating to synthetic pathways. The present work indicates that the addition of carbon-28 might be one of the last steps in the biochemical sequence leading to C_{28}-sterols. In the biosynthesis of these sterols from acetate, it appears that cholesterol is an earlier product. This theory may offer an explanation to one of our earlier observations: When clams were injected with acetate-2-C^{14} the major part of the radioactivity, subsequently found in the non-saponifiable fraction, resided in a sterol presumed to be cholesterol, but very little activity was found in the 24-methylenecholesterol fraction. Possibly another explanation is provided by Clark and Bloch's concept (10) of a differentiation in sterols of the hide beetle into two functional types, viz. structural and metabolic, each with an implied different

rate of turnover.

Tschesche and Korte (11) have advanced a theory concerning the mode of formation of C_{28}- and C_{29}-sterols in plants. According to these authors, the activation of C-24 by a system consisting of a C-22 and a C-25 double bond (Fig. 1) would facilitate the addition of one or more carbon units at C-24.

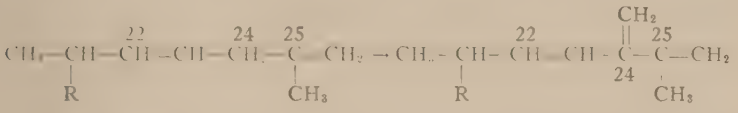


FIG. 1.

Initially a C-22,24(28),25 triunsaturated sterol would conceivably be formed which, by the saturation of appropriate double bonds, would give rise to all known C_{28}- and C_{29}-sterols. If the theory is applied to the synthesis of sterols in clams, saturation of the C-22 and C-25 double bonds would result in the formation of 24-methylenecholesterol. The observations that radioactive cholesterol can be converted by the same animal into C-22 and C-25 unsaturated C_{27}-sterols (6), and into a C-24 (28) unsaturated C_{28}-sterol, together give support to this theory.

Acknowledgments

We are greatly indebted to Dr. Franz Sondheimer, The Weizmann Institute of Science, Rehovoth, Israel, for supplying us with a sample of 21-nor-20-ketocholesteryl acetate and 20-dehydrocholesteryl acetate; to Dr. C. L. Sweeley, University of Pittsburg, for carrying out the gas chromatographic analysis; to Dr. D. B. Quayle, Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C., for supplying the clams; and to Dr. M. Newman, Curator, Vancouver Public Aquarium, for supplying facilities for holding the clams in captivity.

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NOTES ON POLYCHAETA FROM CALIFORNIA TO PERU[1]

E. Berkeley and C. Berkeley

Abstract

Twenty-two species of Polychaeta are recorded, of which eleven were taken in the plankton, but some of these are certainly swimming forms of benthic species. The remainder were either dredged, collected intertidally, or by diving. Thirteen species come from Peru, six from California, and one each from Mexico, Panama, and Cocos Island. Three of them are new to science.

Introduction

This paper is essentially a continuation of our contribution of 1960 (9) on olychaeta from the same region, except that this is now extended to Peru. wo of the collectors who sent us the material which formed the subject of ie previous paper, Mr. W. L. Klawe, of the Inter-American Tuna Commission, id Mr. H. Arai, formerly of the University of California, Los Angeles, have ontributed to the present collection. Dr. M. Lieberman, of the University California College of Engineering, Richmond, California, who is now in harge of the invertebrate investigations started by Mr. W. A. Newman in an Francisco Bay, supplied us with further examples from that region, and Ir. J. H. McLean, of the Hopkins Marine Station, Pacific Grove, California, as sent us material, collected in the course of diving operations, from Carmel anyon. Lastly, we received an interesting collection of pelagic specimens ken off the coast of Peru from Dr. P. N. Sund, of the University of Wash-gton, Seattle. We are grateful for having been privileged to examine these ollections. All of them included species which already have been recorded id adequately described from the region dealt with in this paper and, there-re, these are omitted here. We include only those species which are either ew to science or have other noteworthy characteristics. The individual ollectors are indicated in the text by initials, as in our previous paper.

Polynoidae

Malmgrenia nesiotes (Chamberlin). Chamberlin (11), as *Polynoe*, Hartman (19) (revision of type)

A single specimen in two pieces, measuring jointly 8 mm in length, agrees mpletely with Chamberlin's (11) description of the type. Collected inter-dally at Mancora, Peru (W.L.K.). Hartman, on re-examining the type, ssigns it to *Malmgrenia*, in which we concur.

The prostomium was so "shrunken" in the type that Chamberlain could ot fully describe it. It is considerably longer than wide. The anterior, and uch larger, pair of eyes are on the median transverse line and the posterior air on the posterior margin. This is the first record of the species since the iginal one, which was from Santa Margarita Island, Lower California.

[1]Manuscript received June 19, 1961.
Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, C.

Sigalionidae

Pholoë minuta (Fabricius). Fauvel (15)

A single example from San Francisco Bay, dredged in 20 ft (M.L.), measu about 12 mm and consists of 51 setigers. This is the first record of the spec on the west coast of North America south of Oregon (23).

Pettibone (29) regards *P. tuberculata* Southern as a synonym of this speci We had formerly (4) doubted the synonymy. Southward (33) has shown collections from the Isle of Man that the characters on which differentiati had been based intergrade, and we have recently confirmed this in a la gathering from the Arctic.

Tomopteridae

Tomopteris cavallii Rosa. Fauvel (15), Dales (13)

Two specimens from 4° 31′ S. lat., 82° 18′ W. long. in a tow at 15 m (P.N.S agree with Fauvel's and Dales' descriptions and with specimens record by us (7) from the coast of British Columbia. Dales (13) found the spec widely, but sparsely, distributed in surface waters off the Californian coa The present records are from the coast of Peru near the northern extremi Rosa found it near Valparaiso, Chile. The distribution in the northe Pacific is thus a wide one.

Nereidae

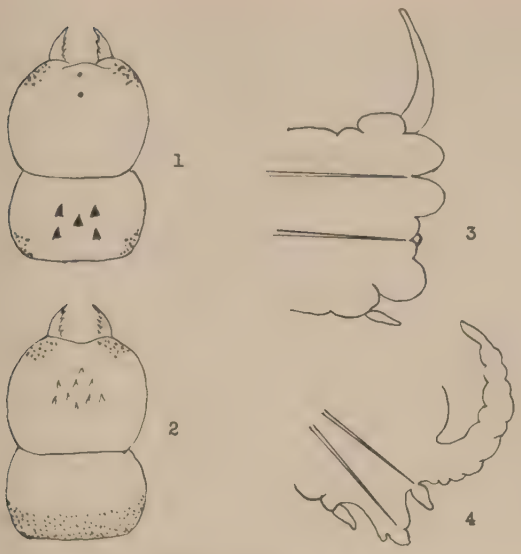
Platynereis polyscalma Chamberlin. Chamberlin (11), Hartman (20)

A number of specimens of the atokous form of this species collected int tidally at Mancora, Peru (W.L.K.). This was first described from Low California by Hartman (20) and has not since been recorded. Epitokes ha been taken from southerly latitudes, but the present record extends the kno distribution of the atokous form.

Nereis* (*Neanthes*) *mancorae n. sp.

Six specimens varying in length from 5 to 20 mm and from 0.5 to 1 m wide, from Mancora, Peru. Intertidal (W.L.K.). No color remains in t specimens. The prostomium is longer than wide. It carries two pairs of ey the anterior pair the larger, both pairs posterior to the median transverse li There are three pairs of short tentacular cirri and a fourth pair (the mo posterior) more than twice as long, reaching to the fifth setiger. The ja have 9 or 10 teeth. Paragnaths as follows: I = 2 medium-sized cones tandem; II, III, IV = 5 or 6 irregular groups of variable teeth more or l in lines; V = 1 large cone; VI = 2 large cones in tandem; VII and VIII 5 or 6 irregular rows of small cones (Figs. 1 and 2). The dorsal cirri exte well beyond the lobes of the feet throughout the body (Fig. 3), and towar the posterior end become still longer and very heavy (Fig. 4). The parapo have two lobes in each ramus. In the posterior region they are, howev much modified (Fig. 4). Notopodial setae throughout the body are all hom gomph spinigers; in the neuropodium there are a few heterogomph spinig in the dorsal bundle, the remainder being falcigers with finely toothed blad

The form is differentiated from other species of *Neanthes* by the arran ment of the paragnaths and the very long and heavy dorsal cirri in the p terior region.



FIGS. 1-4. *Nereis* (*Neanthes*) *mancorae* n. sp. Fig. 1. Proboscis, dorsal view. Fig. 2. Proboscis, ventral view. Fig. 3. Median parapodium. Fig. 4. Posterior parapodium.

Phyllodocidae

Phyllodoce (*Anaitides*) *groenlandica* Oersted. Fauvel (15)

A single specimen taken over kelp beds at a night light at La Jolla, S. California (W.L.K.). This is a typically northern form and has not been recorded previously from California. Treadwell (34), however, records it from Coiba Island, Panama. His specimen was also taken at a night light.

Lopadorhynchus brevis Grube. Wesenberg-Lund (37), Berkeley and Berkeley (8)

A single specimen taken at 8° 00′ N. lat., 91° 32′ W. long. (W.L.K.). We have already recorded the species from approximately the same locality (8), as also does Treadwell (35, as *L. nans* Chamberlin). Chamberlin's record of *L. parvum* (11), which may be the juvenile of *L. brevis* (13), is from a more northerly latitude. Dales (13) records the species from California and summarizes its Atlantic distribution.

Pontodora pelagica Greef. Fauvel (15), Ushakov (36)

A single specimen collected in a 15 m tow at 9° 22′ S. lat., 79° 5′ W. long. (P.N.S.). We recorded the species (10) a considerable distance off the coast of British Columbia (50° 00′ N., 145° 00′ W.) in a vertical haul from 1250 m. The present seems to be the northernmost record, south of that one, at which it has been found. Chamberlin's (11) and Treadwell's (35) records of *Torrea pelagica* (which is a synonym of the present species) are from considerably farther south.

Alciopidae

Torrea candida (Delle Chiaje). Fauvel (15, as *Asterope*)

A small specimen, about 50 mm long as preserved, and several fragments, taken at a night light off Cocos Island (approximately 4° N. lat., 88° W. long.) (W.L.K.). This species is common in the Atlantic and Mediterranean. Dales (13) found it widely, but sparsely, distributed in the northeast Pacific, but not so far south as the present specimens. It does not seem to be known from the south Pacific.

Plotohelmis capitata (Greef). Fauvel (15, as *Rhynchonerella fulgens*)

This is by far the commonest species in Dr. Sund's collection from Peru. It occurs in 10 of his 17 stations which extend from 4° to 9° S. lat. and from 78° to 82° W. long. It has not been recorded from the northeast Pacific where it is replaced by *P. tenuis* Apstein (7, 12, 13).

Callizona nasuta Greef. Wesenberg-Lund (37), Fauvel (15)

Specimens from several of Dr. Sund's stations off Peru, mostly fragmentary, the largest complete example measuring 35 mm. The species is said to be rare in the Atlantic and Mediterranean and the only previous records in the Pacific are those of Izuka (25, as *C. japonica*) and ourselves (10).

Callizona möbii (Apstein). Dales (13, as *Rhynchonerella*)

Two specimens, incomplete posteriorly, each about 20 mm long and 0.5 mm wide, consisting of approximately 50 segments, from 10° 00′ S. lat., 78° 00′ W. long. (P.N.S.). Very little color remains, the intersegmental glands being almost colorless. The first setiger has five simple, curved acicular setae, the second four, and the third three. More posteriorly they are straight and fewer. The species has been reported from the Mediterranean (1) and north Atlantic (37). Dales records it from the northeast Pacific (13, as *Rhynchonerella*). The present record is the first from the south Pacific.

Pilargiidae

Ancistrosyllis bassi Hartman. Hartman (21)

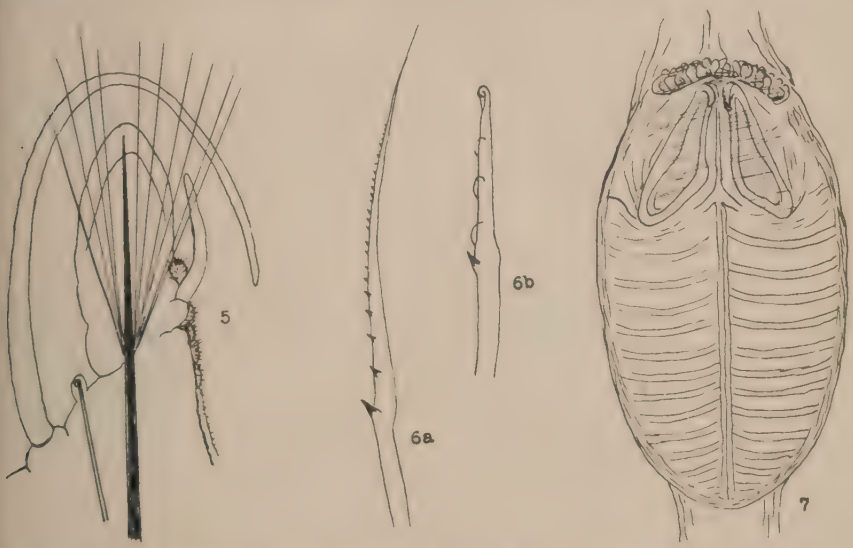
A single specimen dredged in 20 ft in San Francisco Bay, N. California (M.L.). This is the second record from this locality, which was one of the first from which one of the original specimens was obtained (21). It has since been taken in San Pedro Basin (22) and in Newport Bay (2), both in S. California.

Ancistrosyllis longicirrata n. sp.

A single specimen 10 mm long and about 1 mm wide (excluding setae), consisting of 35 segments, from 5° 55′ S. lat., 82° 2′ W. long. (P.N.S.). The prostomium is notched anteriorly, thickened dorsally, and rounded laterally. It bears three antennae, the median four times the length of the prostomium, the laterals about half the length of the median one; and has a pair of rather large eyes placed laterally close to the posterior edge. The palps are biarticulate, the palpostyles small.

The peristomium carries the first pair of tentacular cirri; the second segment (first setiger) the second pair; all are approximately the same length,

about equal to that of the median antenna. At the third segment the parapodia are almost fully developed. Thereafter their length is about that of the body-width and their width at the base about a sixth of their length. The most conspicuous feature of the parapodium is the great length of the dorsal cirrus, more than twice that of the parapodium itself. The length of the ventral cirrus is only about a fifth that of the dorsal cirrus. The parapodium is subbiramous, the notopodium a very small lobe. There is a row of ciliated prominences on the lower edge of the foot which terminates in a rather large stalked ciliated papilla emerging between the ventral cirrus and the base of the parapodial lobes (Fig. 5).



FIGS. 5–7. *Ancistrosyllis longicirrata* n. sp. Fig. 5. Median parapodium. Fig. 6*a*. Seta from dorsal side of group. Fig. 6*b*. Seta from ventral side of group. Fig. 7. Pharynx.

Setae are confined to the ventral ramus and project between terminal lobes. All are simple. The majority are very long and fine with a thickening halfway up the shaft, beyond which are serrations, the first of which are heavy and widely spaced (Fig. 6*a*). On the ventral side of the group there are three to five shorter setae which terminate in small hooks (Fig. 6*b*). The neuraciculum is heavy and protrudes with the setae. The notaciculum is much finer and ends in a small, round hook. Neither large heavy hooks nor heavy spines, such as appear, dorsal to the dorsal cirrus, in most species of *Ancistrosyllis*, are present. The pygidium ends in three small lobes, the median of which bears a pair of slender cirri. There is a well-developed muscular pharynx which encloses a pair of processes which appear to be jaws. (The detailed structure could not be made out without dissection.) Its anterior end terminates in a ring of papillae (Fig. 7).

The species of *Ancistrosyllis* hitherto described (except the extremely small and problematical *A. cingulata* of Korschelt (15, p. 251)) have been benthic.

The occurrence of the present species in the plankton and the presence of ciliated pads on the parapodia suggest that it may be a juvenile of a bottom-living form. It clearly belongs to the group characterized by long appendages (*A. constricta*, *A. robusta*, *A. tentaculata*, and *A. bassi*), but in none of these do the appendages attain the length of those of the present species, nor have jaw-like structures been described in any of them. Moreover, the form of the setae and the absence of dorsal spines are characteristics by which the present species is differentiated.

We follow Hartman (21) rather hesitantly in placing the genus *Ancistrosyllis* in the family Pilargiidae rather than in the Hesionidae, with which it was formerly classified. Hartman regards the transfer desirable because in *Ancistrosyllis* the setae are all simple, while in other genera ascribed to Hesionidae this is not the case. In the present species this character is combined with others, the long dorsal cirrus, the subbiramous foot, the presence of "jaws", and the absence of dorsal spines or hooks, which seem to ally it more closely with the Hesionidae.

The type remains for the present in the authors' collection.

Spionidae

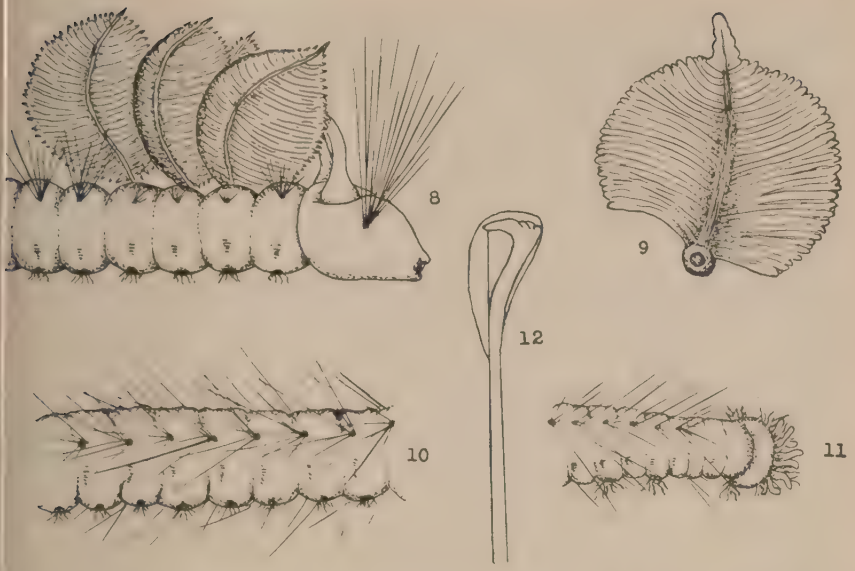
Prionospio ornata n. sp. Larvae

Two collections, consisting jointly of about 75 examples, taken in a 15 m tow at 8° 17′ S. lat., 79° 09′ W. long., and at 8° 50′ S. lat., 78° 50′ W. long. Also several incomplete specimens and fragments from 10° 36′ S. lat., 78° 02′ W. long. (P.N.S.). In all these cases the collector states that many more specimens were present in the plankton samples from which they were isolated.

The longest complete individual measures 8 mm and has 48 setigers, the average length being about 5 mm. They are thus unusually large for Spionid larvae (cf. 18). The general body color, as preserved, is a uniform, very pale, red. There are no markings. On the dorsal side the prostomium is seen as a rounded projection carrying a pair of small, dark, eyespots, but these are covered in a lateral view by the extension forward of the peristomium which bears a pair of grooved tentacular cirri and the first bunch of long larval setae. From the 2nd to the 8th setiger there are dorsally on each side a bunch of larval setae and, ventrally, of short, coarser capillaries. Branchiae are carried on the 2nd, 3rd, and 4th setigers (Fig. 8). These are the outstandingly distinctive characters of the species. Each branchia has two double rows of pinnae, which at their longest are so long that the branchia, when extended and of maximum size, is almost circular in outline (Fig. 9). The pinnae of adjacent branchiae tend to intersperse, giving the whole branchial region a striking bushy appearance.

Posteriorly to the 9th setiger and to the end of the body there are bunches of larval setae dorsally and both crotchets and short capillaries ventrally. The body surface is lightly ciliated throughout and, in addition, there are heavily ciliated protuberances ventrally (Fig. 10). The anal extremity is surrounded by a ring of cirri (Fig. 11). The crotchets are of the characteristic *Prionospio* type with a crest of three small teeth (Fig. 12).

We feel justified in designating these larval forms as a new species despite the fact that we do not know the corresponding adults, because the form of



FIGS. 8-12. *Prionospio ornata* n. sp. Fig. 8. Anterior region. Fig. 9. Branchia. Fig. 10. Median region. Fig. 11. Posterior region. Fig. 12. Crotchet.

he branchiae appears to be unlike that of any recorded *Prionospio*, adult or arval. This character seems to vary very little as between adult and larval spionids (cf. 18). Moreover the size with maintenance of larval characters s outstanding among recorded Spionid species. A comparison of the characters of the present specimens with those of adult species of *Prionospio* suggests *P. pinnata* as the nearest ally, but the two species are differentiated not only by the form of the branchiae, but also by the absence of the vertical extension of the flaps of the peristomium and its fusion dorsally with the first setiger which characterize *P. pinnata*. So far as we know the larva of the latter pecies has not been described.

Cirratulidae

Audouinia polytricha (Schmarda). Ehlers (14), Monro (26, 27)

Three specimens, the first 75 mm long and 4 mm wide, the second the same length but 6 mm wide, the third 85 mm long and 6 mm wide. All collected at San Carlos Bay, Sonora, Mexico, intertidally (H.A.).

We agree with Monro (26) in regarding Chamberlin's (11) *Audouinia filigera nesophila* as a synonym of *A. polytricha*. The species agrees with *A. tentaculata* (Montagu), which has been recorded from the same general neighborhood as the present specimens by Rioja (32), in many respects, but differs from it in regard to the incidence of the branchiae and in the presence of finely toothed, curved capillaries throughout the body in *A. polytricha* but not in *A. tentaculata*. This latter character affords the most readily applied differentiation between the present species and most other

members of the genus. These capillaries are described by Ehlers (14) a figured for *A. filigera nesophila* by Chamberlin (11).

Ehlers recorded the species from the coast of Chile, Monro from Panam The present record extends its distribution northward.

Terebellidae

Nicolea zostericola (Oersted). Fauvel (16)

Three specimens about 6 mm long and 1 mm wide in the anterior regi taken in a plankton tow in 15 m at 5° 55′ S. lat., 82° 20′ W. long. (P.N.S Though small, they agree with adult specimens except in the presence three or four short anal cirri. This may be a postlarval character. On t other hand, eggs are present in the bodies, but Herpin (24) has shown th sexual products occur in *N. zostericola* considerably before the adult stage reached.

The species has not been recorded from the south Pacific, but *N. profun* Chamberlin (11), taken in 1036 fathoms in the same region as the prese specimens, approximates to it so far as can be judged from the incomple description. It differs, however, in having 17, instead of 15, thoracic setige

Sabellidae

Branchiomma roulei Gravier. Gravier (17)

Three examples from Mancora, Peru, collected intertidally (W.L.K Other than our own record from Corona del Mar, S. California (3), th species does not seem to have been noted from the Pacific since Gravie original description (17), from Paita, Peru, in the vicinity of the place origin of the present specimens.

Bispira volutacornis (Montagu). Fauvel (16), Rioja (31)

Three small specimens in soft, muddy tubes from 110 ft in Carmel Canyo S. California (J.H.M.). They agree with Fauvel's (16) and Rioja's (3 descriptions except that the ventral lobes of the collar are flattened a extended forward, possibly as a result of fixation in their tubes. The speci is new to the eastern Pacific. It differs from *Volutacornis pacifica*, describ by us from the coast of British Columbia (6, as *Bispira volutacornis* va *pacifica*), in the formation of the collar.

Serpulidae

Vermiliopsis infundibulum (Phillipi). Rioja (31), Fauvel (16)

Metavermilia multiannulata Moore. Moore (28), Rioja (32, as *Vermiliopsis*)

Several examples, from Carmel Canyon, S. California, in 80–160 ft (J.H.M agree with the descriptions of this species. They are about 20 mm long witho the branchiae, and consist of about 80 segments. The operculum, with multiannular "chitinous" cap, is the most outstanding and distinctive featu Specimens with a dozen, or more, opercular plates occur in the collection, b the terminal ones are readily broken off. The large, flaring collar segment also very characteristic. The thoracic membrane is folded rather closely ov the thorax in the present specimens, doubtless because of their having be preserved in their tubes.

The species has not been recorded from the Pacific as *V. infundibulum*, ut we can see no reason to doubt its identity with *Metavermillia multian-ulata* (Moore) (28). Rioja's redescription (32) confirms this synonymy. He ientions certain differences in tube formation between his specimens from lexico and Moore's from S. California, but Moore's description of it is very rief and based upon a fragment. In the present collection there is considerable ariation between the tubes. In general they share the characters shown by auvel (16, fig. 124*c*) for *V. infundibulum* and those of the relatively smooth ube shown by Rioja (32, pl. 9, fig. 28) for *V. multiannulata*. Most of them re heavily transversely wrinkled, have two or more irregular longitudinal dges, one or two wide flanges, and a flaring mouth. In some cases two or ore tubes were adherent to one another, suggesting that they had grown nder crowded conditions.

The species is known from the Atlantic, Mediterranean, and S. California.

hitinopoma groenlandica (Mörch). Pixell (30), Berkeley and Berkeley (5)

Several specimens of this species, collected at approximately 80 ft in Carmel anyon, S. California (J.H.M.), agree with Pixell's (30) and our own des-iptions. Most previous records of it (frequently under the synonym *C. bricii* Levinsen) are from northern latitudes. From the northeast Pacific is known only from Vancouver Island (30, 5).

aradexiospira vitreus (Fabricius). Pixell (30), Berkeley and Berkeley (5)

Many specimens on shells collected in approximately 80 ft in Carmel anyon, S. California (J.H.M.). A typically northern species. Not recorded reviously on the west coast of North America south of Vancouver Island 30, 5).

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SOME OBSERVATIONS CONCERNING EXPERIMENTAL APPLICATION OF OBJECTIVE QUALITY TESTS TO WEST COAST FISH

by

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It would seem that there are hardly any fisheries technologists who have not spent at least some time working on that elusive subject of objective tests for fish quality. One could go further and state with some truthfulness at least that there is no subject upon which so much effort has been expended by so many fisheries technologists with so few concrete results. The reasons for this are quite obvious. As an example, those of us who have worked with, or are aware of, the bacteriological research which has been carried out on milk preservation will recall the complexity of this problem. No one objective test is available which will enable us to judge with absolute confidence the quality of milk received at a milk plant. With milk, a number of tests have been used from time to time, or are still in force, and these include direct and viable bacterial counts, counts of colon organisms, methylene blue, resazurin or other dye reduction tests, determinations of leucocytes as indications of mastitis contamination, etc. Organoleptic grading is also necessary. Milk is a relatively homogeneous material in comparison with fish.

Fish spoilage is of two important general categories, namely, bacterial and enzymic spoilage and spoilage occurring in frozen fish. Though deteriorations may occur in canned fish products, these are not very well understood and are undoubtedly not nearly so important as the first two mentioned spoilage categories. Too many workers have disregarded spoilage of frozen fish and assumed, in their search for objective quality tests, that they are dealing solely with bacteriological spoilage of fresh fish. The purpose of this present paper is to discuss very briefly some of the research which could have an application or a bearing on fishery inspection problems as they may arise on the Pacific coast of North America. The problems in this area are indeed very different from those on the eastern coasts of Canada. There are a number of important varieties of food fish such as five species of Pacific salmon, black cod, halibut, lingcod, various species of soles, graycod, etc. Presently these fish are fished and treated in quite diverse fashion. Thus, some are eviscerated and some are not eviscerated, some are iced and some are not iced, some are transported under ideal conditions and others under poor conditions. There is a large important canning industry and a comparatively small fresh fillet industry. Much of the fish which is frozen is frozen round and is glazed, though there has been an increasing volume of frozen filleted fish. Presently, with the information and background we have available, it would

seem a fairly hopeless task to attempt to find any one test for bacteriological freshness for these fish, let alone a single test which would cover all types of spoilage. An outline of some of the past research which has been done and which can be considered directly related to this subject follows.

The 1937 mimeographed Annual Report of the then Pacific Fisheries Experimental Station contained summaries concerning various bacteriological, chemical and biochemical studies which were carried out in an attempt to discover good objective tests for halibut quality. A number of different methods were studied with, in general, rather inconclusive results. Thus, it was found that the viable bacterial population of the poke muscle of halibut increased very much more rapidly than that of the muscle near the anal region during storage in ice, and there was a very poor agreement between trimethylamine tests and viable bacterial counts. A determination of the hydrogen sulphide content of halibut muscle from different regions indicated that a test for this substance would be absolutely worthless since there was no tendency for an increase due to storage, the values being scattered throughout. No correlation between the acid soluble inorganic phosphorus of the muscle and storage time was found. Other tests included studies with a textometer as indication of firmness of the flesh, determinations of total volatile nitrogen, and studies of Stansby's A and B values. In general, these tests did not indicate good correlations with storage time of halibut in ice, or insufficient tests were conducted to establish definite correlations.

Following this series of experiments, objective tests for quality have been carried out from time to time at the same station, usually using such tests as criteria of quality in experimental work dealing with preservation of different species of fish. The following is a brief summary of the results.

1938. In studies on the storage of lightly smoked fish it was found that there was no good correlation between viable bacterial counts of lightly smoked fillets of cod, halibut, and salmon, and the trimethylamine values of the same stored samples. These studies indicated that bacterial counts could give quite as good, or perhaps better, indication of spoilage than trimethylamine tests.

1939. Experiments with iced, eviscerated halibut showed that during storage viable bacteria increased very much more markedly in the poke muscle (visceral cavity muscle) than in the belly muscle. Trimethylamine also increased much more markedly in this region. On the other hand, with individual fish there was no good correlation between either the bacterial counts and trimethylamine values, or between either of these and the length of time fish were held in the ice. The values obtained were scattered and irregular, indicating that if either of these methods is ever to be used as an indication of quality, quite large numbers of samples of fish would have to be taken. This itself creates a problem when one is dealing with large loads of comparatively expensive fish of very different post-mortem condition. Rather similar results were obtained in an experiment with eviscerated iced black cod. In both the above experiments it was shown that benzoic acid suppressed development of trimethylamine almost entirely without having much effect on the development of viable bacteria.

1942. Experiments showed that the trimethylamine and total volatile basic nitrogen content of canned herring was practically the same whether it was prepared from fresh herring, herring stored up to seven days at 40°F., or canned herring which had been rejected by an inspection laboratory.

1943. In experiments which were carried out in connection with evolving a method of counting bacteria in fish flesh directly, it was found that with whole fish and fillets there was a reasonably good agreement between direct bacterial counts and counts obtained by the usual laboratory viable counting procedures. The direct count method was shown to be unsatisfactory where there were less than 1×10^5 organisms per gram of muscle. It was shown that there was absolutely no relation between the bacterial counts obtained before incubating fish muscle for 24 hours at 15°C. and the counts obtained after such incubation. Thus, ratios of counts obtained after such incubation with the counts obtained before incubation varied from 440 to 550 at the commencement of the tests with the fresh fish to from 490 to 2500 after storage for one day at 15°C. In one instance a ratio of 28,000 was obtained after a sample had been stored for four days. This general result is due presumably to the lag or initial stationary phase phenomena commonly experienced in bacterial cultures. Thus, though the tendency to multiply under more favourable temperatures definitely increases in cultures held for a day or so, the actual initial counts in these cultures may not be very different. This finding is of distinct importance in any inspection program since it indicates definitely that a comparatively low count obtained on a fresh fillet may not be related well to its potential keeping quality. The reporter therefore favours an inspection method which would involve if possible an incubation technique for samples of fish; incubation for one day at, say, 10°C. would definitely indicate the potential keeping quality of suspected or other samples. *Direct bacterial counts could be readily carried out on such incubated samples and the results obtained within a few minutes!*

At about this time a small survey was made of a number of different retail stores which sold fish in Vancouver. About 35 samples of different types of fish were tested bacteriologically using the direct count method, and it was found that 25 per cent of the samples had counts of less than one million bacteria per gram, 50 per cent had counts of between one and 10 million bacteria per gram, and the remaining 25 per cent had counts of over 10 million bacteria per gram. While this survey was too limited to indicate definitely the quality of market fish which could be expected in this area, it did show that very little fish of really high bacteriological quality was available to the consumer. Undoubtedly these counts would be very much higher by the time many consumers had taken home the fish and cooked it perhaps the following day.

1944. A number of experiments were carried out in order to assess the comparative value of direct bacterial counts and trimethylamine tests in assessing quality of iced eviscerated lemon sole, brill and graycod, and of fillets of lemon sole, graycod and defrosted lingcod. The results indicated that while there was some correlation between the bacterial counts and the trimethylamine increase there were a good many instances where agreement was not at all good. In extreme

cases trimethylamine values of only one or two were obtained with fish that was organoleptically quite stale and had extremely high bacterial counts, though these were not usual cases.

1945. Tests were carried out with iced lemon sole and herring in order to determine the value of direct bacterial counts and carbon dioxide values in determining freshness. While the direct bacterial counts increased fairly steadily with time, carbon dioxide values at first tended to decrease and then to increase again, indicating that they were completely valueless from the point of view of determining spoilage.

1947. Thorough studies were made in order to assess the comparative value of a number of germicides which might be used in disinfecting fish vessels and premises. Compounds studied included a number of quaternary ammonium substances, certain phenolic substances and phenol itself, formaldehyde and chloramine T and sodium hypochlorite. The results indicated that in general sodium hypochlorite was the most effective and cheapest agent but that it is extremely corrosive. Certain of the quaternary ammonium compounds were extremely effective and of course not corrosive. In certain instances the addition of sodium nitrite was found to inhibit corrosion of iron surfaces very effectively, but of course this was not true with hypochlorites. Unpublished studies indicated that germicidal agents have little value in disinfection of fish vessels and premises unless there is preliminary cleaning. The only really effective way of cleaning dirty wooden surfaces is by a detergent under steam pressure, and after such treatment, if carried out properly, a germicide may not be very necessary.

1949. The effect of flesh acidity on bacterial numbers and trimethylamine content of the flesh of species of salmon and cod was investigated. There was some indication that the acidity of the flesh tended to suppress trimethylamine formation but the evidence was not very consistent. On the other hand, samples of fillets were obtained where the trimethylamine values were between two and three and the fish was quite stale. *In half the samples which had bacterial counts of between 100 and 1000 million bacteria per gram of flesh, the trimethylamine value was less than 2.0.*

1949. The effect of mechanical washing of fish in the keeping quality and bacterial condition of a number of eviscerated fish was studied. The washing procedure caused a marked improvement in keeping quality.

Oxidative rancidity. Oxidative rancidity is one of the most serious causes of deterioration of frozen fatty fish and even non-fatty fish and consequently is very important to west coast fish since quite a large amount is frozen. It is for this reason that, since about 1944, the Vancouver station has conducted a good deal of research into the deterioration of cold-stored fish and the effect of antioxidants, gas storage, packaging and glazing procedures in hindering development of oxidative rancidity. In this work peroxide value determinations and, more recently, thiobarbituric acid values, have been used as objective criteria of quality. Though these methods are indeed useful in *comparative* experiments using the same material, it is highly doubtful if they would be a great deal of use in determining the condition of frozen fish taken from cold storages. In any event, a very

large amount of research would have to be carried out before any definite decision could be made concerning the value of these tests. As an example, a peroxide value would probably have to be correlated with the oil content of the fish, for it would seem highly probable that a very fatty fish might become organoleptically rancid when the fat developed a fairly low peroxide value while a similar value found for a comparatively non-fatty fish might not indicate oxidative rancidity. Protein denaturation is another problem which undoubtedly is very great in the storage of west coast fish, since so much of the catch is obtained during comparatively short fishing seasons and the storage times are often prolonged and may be in some instances over a year. Unfortunately, presently there does not appear to be any good test which will indicate the degree of denaturation of fish. The hope that the decrease in salt solubility of myosin might be of some value in this connection must now be seriously questioned in view of the recent European finding that in cold-stored fish held at comparatively low temperatures organoleptic toughening may occur without an important decrease in the solubility of muscle myosin in cold salt solutions of fairly high ionic strength.

Objective tests for quality of Pacific coast fish have been carried out in several laboratories in California and Washington and also in Oregon. A test for total volatile reducing substances (VRS) has been used experimentally in a few localities and seems to offer some promise. However, it is somewhat time-consuming, and as far as I am aware is not used to any extent at the present time. Work at this Station indicated that it was of no value for certain of our salmon. Work carried out at the University of Washington has indicated that the trimethylamine tests may be of some value in determining the quality of certain species of Pacific sole. Tests carried out several years ago in a Seattle laboratory indicated that measurement of surface pH was of no real value in assessing quality of certain Pacific coast fish.

In conclusion, the reporter feels that the best single test for bacteriological quality of fish would be a determination of the total bacterial population. Determination of any one chemical constituent, and even of mixed chemical constituents, should be less reliable in the long run since it is unlikely that all bacteria present would form the given chemical substance, and thus the amount of the substance would naturally depend on the proportion of the organisms present which formed that substance. It is felt that there is much to be gained by an incubation technique since, as expressed above, this indicates the potential keeping quality of a given fish sample, and this is the important thing from the point of view of the consumer. However, even bacteriological tests are subject to serious drawbacks for a number of reasons, and it is doubtful whether they could be used as absolute criteria of quality. For example, they do not take into account very important grading factors such as chalkiness in halibut, effect of fattiness as in spawning migration on quality, parasitism, etc. This document is not intended to be discouraging but has been prepared specifically with the idea of pointing out some of the difficulties which may arise in attempting to arrive at reliable objective tests for fish quality.

OZONOLYSIS OF UNSATURATED FATTY ACIDS

I. OZONOLYSIS OF OLEIC ACID

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F.R.B. No. 662

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ABSTRACT

Ozonolysis of oleic acid in methanol, a reacting solvent, with subsequent decomposition of the ozonide products by hydrogen peroxide in formic acid, gives yields of principal dicarboxylic acid fission products exceeding 95% with a minimum of secondary acidic products. The method is highly reproducible and offers unique advantages in the total recovery of the dicarboxylic acids and the elimination of peroxidic materials. The principal non-acidic by-products were tentatively identified as the C_8 alcohols and their formyl esters.

INTRODUCTION

The study of the position of the ethylenic double bonds in long-chain fatty ac important in connection with the isolation of various naturally occurring fatty and even more important in fields where the chemical treatment of the fatty ac partial hydrogenation, alkali fusion, or other prototropic reactions leads to the form of positional isomers. In the former instance, any reasonable yield of principal f products resulting from the scission of the double bond is satisfactory for bond loc particularly as naturally occurring fatty acids are usually homogeneous in bond po and impurities will differ enough structurally to be recognized as such. In the ca mixtures of positional isomers, the over-all yield is less important than two other fa which must be considered. Firstly, the occurrence of abnormal fission products su lower homologues of the principal fission products must be reduced to a minimum secondly, the method of recovery and analysis of the products must be such tha analysis reflects the true proportions of these products.

Scission of the double bonds is usually achieved by oxidative fission. Some wor been done to give aldehydes as primary products (1–9), but the more extensive applic of this technique has always been in the direction of obtaining the more tractable fission products. Ozonolysis is an attractive technique owing to the complete rea of ozone with the starting material (8) and the simplicity of the reagents and proce generally adopted. The chief disadvantages, other than the necessity of an ozone rator, have always been the nominal yields (70–85%) (10–16) of primary acidic f products, the associated formation of carbonyl, peroxidic, and polymeric mat (1, 7, 17), and the presence of one or more secondary acidic oxidation products latter can cause considerable uncertainty where minor amounts of positional iso are being determined, while by-products hinder both separation and analysis o products (10, 18). Certain procedures do give high yields of products, but the oxide oxidation method of Asinger (3, 12, 19) and the procedure of Klenk and Bo (20) are somewhat complex, while that of Pryde *et al.* (8) yields aldehydes as pri products.

The most common alternatively employed oxidizing agent, potassium permanga also gives only moderate yields of acidic products and considerable secondary oxid (11, 12, 18, 21–24). The recently introduced permanganate-catalyzed periodate m of von Rudloff (3, 25–28) is much more attractive owing to the simplicity of the m

[1]*Manuscript received March 20, 1961.*
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d the very high yields of primary products. These techniques do suffer from a dis-
vantage in that recovery of the primary products, in particular the dicarboxylic acids,
ıst be made by extraction from a highly mineralized aqueous solution, with consequent
k of losses of the more soluble lower dicarboxylic acids. In the past this factor has led
erroneous results in determining the relative proportions of positionally isomeric
uble bonds (13, 24, 29, 30).

If ozonolysis is to be considered as a useful method for the sensitive determination
double-bond position in long-chain fatty acids it must satisfy the following require-
ents:

1. the method should have a high yield of primary products and should give rise to the
ast possible amounts and variety of "abnormal" products;
2. the procedure of working-up should give the highest possible recoveries of related
oducts even when these have differing physical properties;
3. the method should be simple and reproducible;
4. the method of analysis should be sensitive and accurate.

It was deemed desirable that the principal products should be acidic in order to
pitalize on the stability and low volatility of the desirable dicarboxylic acids. The
ethod outlined below, based on ozonolysis in methanol, a reacting solvent, with de-
mposition of the ozonide product with hydrogen peroxide in formic acid, satisfies the
ove requirements. The yield of principal dicarboxylic acid product averages 95%,
companied by only 2–4% lower homologous dicarboxylic acids and 2–4% other
aterials. The principal non-dicarboxylic acid by-product has been tentatively identified
the alcohol formed from the principal acidic product through the loss of one carbon
om, occurring both as the alcohol and as its formyl ester. Since only volatile solvents
d reagents are employed, and the monocarboxylic acids are removed by steam dis-
lation, total recovery of the dicarboxylic acids present is possible. The method is
atively simple and while some variation is found in the proportion of non-dicarboxylic
oducts to dicarboxylic acids, the ratio of total secondary dicarboxylic acid products
principal dicarboxylic acid product is remarkably stable. The mixture of products is
adily analyzed with a high degree of accuracy by gas liquid chromatography. The
ethanol – formic acid procedure is derived from that employed by Bailey (31, 32) for
e production of adipic acid by ozonolysis of cyclohexene, by Perry (33) in the ozonolysis
norbornylene, and by Pryde *et al.* (8) in the ozonolysis of methyl oleate to give carbonyl
oducts. Methanol has also been employed recently by other workers in the ozonolysis
ld (34–37), although not necessarily in exploitation of the same type of reaction.

EXPERIMENTAL

Pure oleic acid was prepared from filbert oil (38) via the methyl esters by established
ocedures (39, 40). The following properties were determined: iodine value 91.2 (Wijs),
.1 (by precision semimicro hydrogenation (41)) (calc. 89.9); n_D^{25} 1.45785 (cf. n_D^{25}
5792 (42)). Ultraviolet spectrophotometry showed no diene or triene content prior
alkali isomerization* and the latter procedure showed a diene content of 0.1% or less.
as liquid chromatography of the methyl ester showed only an oleate peak.

It has been reported that an oleic acid prepared from olive oil by standard methods
ntained appreciable amounts of positional isomers (29). The present and other studies
3, 14) have confirmed that oleic acid homogeneous in bond position can be readily

*Modification of the method of Vandenheuvel and Richardson (43); 21% KOH in ethylene glycol, effective
ting time 15 minutes.

prepared. The ultimate criterion of purity in all cases must be the analysis of both mo and di-carboxylic acid fission products. Thus the presence of small amounts of sub acid in the dicarboxylic acid product does not signify the presence of octadec-8-e acid unless decanoic acid can be found in the complementary monocarboxylic acids the present studies the occurrence of both mono- and di-carboxylic acid products c taining 10 carbon atoms has been noted in amounts of $\leqslant 0.1\%$. Thus the amount octadec-8- and octadec-10-enoic acids cannot have exceeded this level.

Ozone was furnished by a pyrex corona discharge tube (4). At an oxygen flow of 1.1 l./hr, the ozone content was determined as 2.2% (w/w) by decompositio potassium iodide (44). A dry-ice trap supplemented chemical drying of the oxygen.

1. Ozonolysis in Methanol

Pure oleic acid (ca. 1 g) was weighed in a long-necked, 100-ml standard taper fl Absolute methanol (Fisher Spectrophotometric Grade, 40 ml) was added and the solu was chilled to $-30°$ C and treated with ozone-containing oxygen until complete abs tion ceased. The flask was fitted with a distilling head in order to admit a capillary t (nitrogen) and was connected through a dry-ice trap to a mechanical vacuum pu system. The flask was immersed in water at room temperature and vacuum applie stages until all the methanol was removed and the pressure was less than 1 mm. For acid (98%, 25 ml) was added to dissolve the syrupy residue and hydrogen peroxide (3 50% aqueous solution, 10 mole-equivalents) added. A reflux condenser was immedia fitted to the flask and gentle warming induced an exothermic reaction, on comple of which the mixture was heated for 1 hour on a steam bath. The peroxide and per content fell rapidly below measurable limits.

Water (20 ml) was added and the solution transferred to a continuous steam-distilla unit, a few drops of acetone serving as rinse. In this case, 200 ml of distillate was colle for recovery of the nonanoic and associated monocarboxylic acid products. The carboxylic acid solution was transferred to a distillation unit and evaporated nearl dryness under aspirator vacuum. Toluene was added in small lots (ca. 10 ml) and remo under reduced pressure until the distillate was free of water and formic acid (45). Fur vacuum treatment eliminated residual toluene. The product was dissolved in ace (100 ml) and aliquots were taken for determination of yield by weight and titrat The balance of the material was freed of solvent and esterified with distilled diazometh (18) in ether solution. The ether was removed and the product examined by gas–li chromatography. The results are presented in Table I.

The steam distillate was extracted with hexane (100 ml). The hexane solution dried over sodium sulphate, and after removal of solvent the monocarboxylic a were converted to methyl esters with diazomethane. Analysis of these methyl este presented in Table II.

The methanol in the cold trap was diluted with water and extracted with hex The bulk of the solvent was removed and the remaining solution examined by gas–li chromatography. Only traces of nonaldehyde were observed.

2. Ozonolysis in Acetic Acid and Other Solvents

Ozonolyses were carried out as described above employing 40 ml of a mixture of ac acid and methyl formate (3:1), acetone, or methyl formate, with cooling in an ice-w bath. After complete ozone absorption had ceased, aqueous hydrogen peroxide (3 5 ml) was added and the flask was fitted with a reflux condenser. After standing overn at room temperature, the mixtures were heated on a steam bath for 1 hour.

TABLE I

Dicarboxylic acids obtained from ozonolysis of oleic acid and control oxidations

Experiment			% yield		Weight% dicarboxylic acid composition*							% other material‡
un	Material	Solvent	Weight†	Titration†	C_9	C_8	C_7	C_6	C_5	C_4	C_3	
-A	Oleic	CH_3CO_2H	96.2	77.5	70.6	7.1	6.1	3.8	4.7	6.9	0.8	24.1‖
-B	Oleic	$(CH_3)_2CO$	83	73	86.8	8.2	1.2	1.1	1.1	1.6	—	20.7§
-A	Oleic	MeOH	100	93.5	97.2	1.9	0.4	0.2	0.2	0.1	—	5.6‖
-B	Oleic	MeOH	100	93.1	97.5	2.2	0.1	0.1	0.1	—	—	3.1‖
-C	Oleic	MeOH	100	95.5	98.7	0.9	0.1	0.1	0.1	0.1	—	1.8‖
-D	Oleic	MeOH	106	100	97.6	1.9	0.1	0.1	0.1	0.1	—	3.9‖
-A	Oleic	MeOH			96.0	2.5	0.6	0.3	0.3	0.3	—	5.4§
-B	Oleic	MeOH			95.4	2.9	0.7	0.4	0.2	0.4	—	4.2§
-C	Oleic	MeOH			97.9	1.5	0.1	0.1	0.2	0.2	—	0.7§
-D	Oleic	MeOH			97.8	1.5	0.2	0.2	0.2	0.1	—	1.5§
-A	Azelaic	HCO_2H	97.7	98.2	98.9	1.0	—	—	0.1	—	—	1.1‖
-A	Azelaic	HCO_2H			98.5	1.2	0.1	—	0.1	0.1	—	1.1§
-B	Azelaic	HCO_2H	98.6	100.3	99.1	0.6	0.1	—	0.1	0.1	—	0.6§

% of total dicarboxylic acids indicated by gas–liquid chromatography.
Calculated as azelaic acid.
% of total volatile materials indicated by gas–liquid chromatography.
Polyester substrate.
Silicone grease substrate.

TABLE II

Monocarboxylic acids from ozonolysis of oleic acid

Run†	% monocarboxylic acids	Weight% monocarboxylic acid composition*			% other material‡
		C_9	C_8	C_7	
2-D	95.8§	98.1	1.6	0.3	4.2
	95.7‖	98.4	1.2	0.4	4.3

*% of total monocarboxylic acids indicated by gas–liquid chromatography.
†Details set forth in Table I.
‡% of total volatile materials indicated by gas–liquid chromatography.
§Silicone grease substrate.
‖Polyester substrate.

Monocarboxylic acids were removed by extraction with hexane (100 ml) after addition water (100 ml). The aqueous solution containing the dicarboxylic acids was freed of lvents and volatile acids as described above, yields were determined, and the product as converted to methyl esters. Gas–liquid chromatographic analyses of the products e presented in Table I for the acetic acid and acetone products. The methyl formate oduct did not differ appreciably from the acetone product.

Control Oxidation of Azelaic Acid

Pure azelaic acid (100% by gas liquid chromatography of the dimethyl ester) was fluxed for 1½ hours with hydrogen peroxide solution in formic acid in the proportions used the ozonolysis procedure. After removal of the solvent the recovery was 97.7% by eight. The esters were examined by gas–liquid chromatography and gave dicarboxylic id chromatograms almost identical with those obtained from ozonolysis (Table I). he only significant feature appears to be the lower amounts of pimelic and adipic acids rmed and the absence of the alcohol by-product.

Control Oxidation of Azelaic Acid Methyl Ester Semialdehyde Acetal

Azelaic acid methyl ester semialdehyde was prepared from methyl oleate by ozonolysis acetic acid and reduction with zinc [8, 9]. After a preliminary distillation, a concentrate

was converted to the acetal by refluxing with methanolic HCl (46). The acetal me ester was treated with sodium bisulphite solution and after removal of solvent purified by preparative gas–liquid chromatography (47). The product was not comple pure (cf. ref. 8), but on treatment with hydrogen peroxide in formic acid, a vigo exothermic reaction took place, and working-up of the products, as in section 2 ab gave a product the gas–liquid chromatogram of which was virtually identical with obtained by oxidation of azelaic acid.

5. Determination of Peroxidic Materials

An aqueous solution of hydrogen peroxide (30%, 5 ml) was added to the solvent u consideration (40 ml) in a round-bottomed standard taper flask fitted with a r condenser. The solution was heated on a steam bath, aliquots being withdrawn at inte for analysis. Determination of hydrogen peroxide was made with ceric sulphate, an peracids, with potassium iodide and sodium thiosulphate (48). Only formic acid sho an appreciable peracid content at any time, and only with formic acid or mixtures taining formic acid was rapid destruction of all active oxygen evident.

6. Gas–Liquid Chromatography

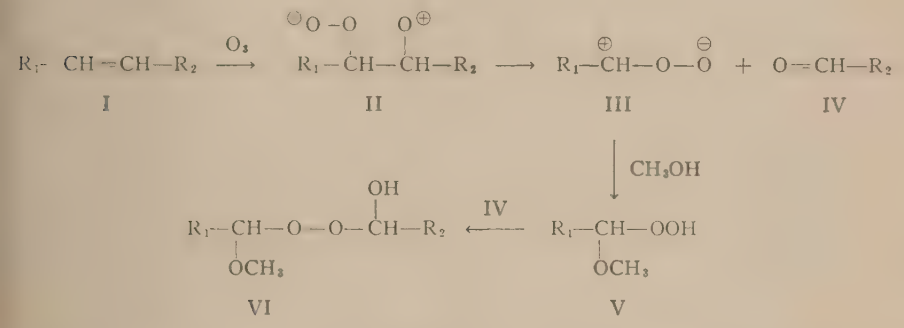
The apparatus employed was a Podbielniak Chromacon. Columns were 10 ft in le and $\frac{1}{4}$ in. in diameter, packed with 30% of either high-vacuum silicone grease (Corning), especially treated (49), or diethylene glycol – adipic acid polyester (LA R-446), on 60–80 mesh Chromosorb. Analyses of monocarboxylic esters were carried at 100° C with hydrogen flow rates of 55 ml/min for the polyester column and 100 ml/ for the silicone column. Dicarboxylic esters were analyzed at 160° with hydrogen rates of 85 ml/min for the polyester column and 38 ml/min for the silicone column. A were determined by a ball-and-disk-type integrator. The errors associated with small peaks ($\sim$0.1%) may be $\pm$30% owing to the few counts available (50).

The monocarboxylic acid chromatograms showed, in addition to minor amount octanoic and heptanoic methyl esters, three other components. The minor one identified as nonanal and the others as octanol and octyl formate, the latter pred nating. These identifications are assigned on the basis of complete coincidence of s additions of authentic materials added to the ozonolysis product, carried out on polyester and silicone substrates.

In addition to a large number of very minor peaks, the dicarboxylic acid ester chro tograms showed one major non-dicarboxylic acid by-product. Methyl 8-hydroxyoctan was not available, but methyl 9-hydroxynonanoate was prepared by the method of Di and Mitchell (51). Calculations of the equivalent chain lengths (52) of methyl 8-hydr octanoate were made from the retention times of methyl 9-hydroxynonanoate. T indicated that the retention times, relative to dimethyl azelate, would be 0.605 on silicone column and 1.16 on the polyester column. Minor peaks were in fact observe 0.610 and 1.20 respectively. The formyl ester of the alcohol methyl ester could be simi shown to have relative retention times, calculated from those of the formyl ester of me 9-hydroxynonanoate, of 0.680 on the silicone grease column and 0.905 on the polye column. These values correlate with the relative retention times of the major dicarboxylic acid product, 0.706 and 0.902 respectively. The value of the relative reten time for the by-product peak on the silicone grease column could not be accura determined as it was overlapped by the dimethyl suberate peak. Mixed runs with s amounts of methyl azelaaldehyde (9) did not coincide with any component on ei silicone or polyester analyses.

DISCUSSION

The Criegee ozonolysis mechanism (1, 53, 54) suggests that the zwitterion (III) in e presence of a reacting solvent, in this case methanol, leads to a methoxy hydro-roxide (V). There is a possibility that this may then react with the aldehyde (IV) to re a hemiperacetal (VI). If the latter step were not complete (cf. ref. 33), some loss volatile aldehyde would be expected when the methanol is removed under vacuum, ereas only a trace of free aldehyde (nonaldehyde) has been observed in this step. eaction of the aldehydes with methanol to give hemiacetals or acetals is also a possi-ity (37).



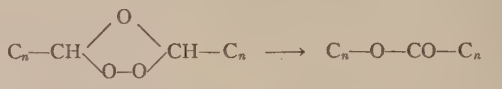
The decomposition of the hydroperoxides, hemiperacetals, hemiacetals etc. is readily complished by treatment with hydrogen peroxide and formic acid, the active agent obably being performic acid (55) formed *in situ*. Peracetic acid has been employed in nilar cases (15, 16, 37). Performic acid decomposes readily on heating, ensuring the pid destruction (31, 32) of all peroxidic materials, to leave inert products. Regardless the nature of the material being oxidized, less than 1% nonaldehyde and only traces azelaic semialdehyde are found in all cases. This can be taken as a reliable indication at oxidation is virtually complete.

The quality of the reagents is significant. Thus results 2-A and 2-B, Table I, where linary reagent grade methanol was employed, show slightly higher amounts of both arboxylic acid and non-dicarboxylic acid by-products than results 2-C and 2-D where ectrophotometric grade methanol was used.

The acidic composition of the monocarboxylic acid fraction (Table II) parallels, in rtain respects, that of the dicarboxylic acid product. However, since the recovery is ly about 90% and losses of lower materials are high, the results are less significant. The close duplication of the ozonolysis results by the control oxidations of azelaic acid d azelaic acid methyl ester semialdehyde acetal (see Table I, also above), not only terms of secondary dicarboxylic by-products, but also partly in the miscellaneous by-oducts, indicates that these materials result from true secondary oxidation by the drogen peroxide in formic acid, and do not form during the ozonolysis step.

The occurrence of the presumed alcohol by-products in both the monocarboxylic d dicarboxylic acid products suggests a symmetrical reaction characteristic of isolated uble bonds, and since they are not found in the control oxidation reactions (see above), eir formation must take place during the ozonolysis or decomposition of the ozonide. vo possible mechanisms may be considered. In the first instance Pasero *et al.* (56–58)

have observed the formation of appreciable amounts of esters during the ozonoly monoethylenic acids, and ascribe this to simple decomposition of the ozonide:



Thus monooctyl azelate is one of the two potential products in the ozonolysis of acid, and in the presence of formic acid and water, might be expected to hydrol transesterify to give both octanol and octyl formate, together with azelaic acid.

Lefort *et al.* (59, 60) have observed the formation of similar esters (C_n—O—CO- when aliphatic peracids are heated, although under anhydrous conditions, suggest complex reaction of peracids during the ozonide decomposition with hydrogen per in formic acid. On the present evidence either mechanism might be applicable.

Complete elimination of by-products is a highly desirable but rarely attained e all oxidative fission procedures. The methanol – formic acid procedure reduces th product level to a point where gas–liquid chromatography is greatly facilitated, wh the same time retaining the advantage of total recovery of the dicarboxylic acids. by-products do not, therefore, detract from the advantages of this technique, com with gas -liquid chromatography, as a quantitative means of analysis of the positi ethylenic double bonds in long-chain monounsaturated fatty acids.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. M. Bannerman for carrying out gas–liquid chr tographic analyses and to Mr. P. M. Jangaard for the ultraviolet and alkali isomeriz studies.

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THE IRRIGATION OF THE GILLS IN FISHES

I. STUDIES OF THE MECHANISM OF BRANCHIAL IRRIGATION[1]

RICHARD L. SAUNDERS[2]

Abstract

A technique which does not interfere with normal respiratory movements was developed for measuring the changes in hydrostatic pressure in the oral and opercular cavities of fish breathing at various intensities. This consisted of inserting polyethylene tubing of small diameter through the cleithrum into the opercular cavity and through the rostrum into the oral cavity. These cannulae were connected with manometers which were used together with optical lever systems to record the time course of changes in hydrostatic pressures in the two components of the branchial pump.

In the white sucker, brown bullhead, and carp the flow of water over the gills is nearly continuous. There are only brief periods when the pressure in the opercular cavities exceeds that in the oral cavity and therefore only brief reversals in flow in which some water may be returned over the gills. The slits between adjacent hemibranchiae of neighboring gills were observed to open rhythmically during moderate and heavy breathing. This observation is contrary to the thesis of many workers that water bathing the gills flows only through the minute pores between adjacent lamellae. Such opening of the gill slits may have a significant effect in the operation of the gills.

Introduction

Branchial irrigation, the passage of water over the gills for the purpose of respiration, is accomplished in teleosts through the active respiratory movements and as a result of the fish's forward movement through the water. This investigation is a comparative study of the active respiratory movements and the mechanisms producing them in three fresh-water teleosts. The time course of changes in hydrostatic pressure was measured and recorded, and an analysis was made of the movements of the gills themselves during their irrigation.

The Branchial Irrigation System

The Branchial Pump

The following works should be consulted by those interested in discussions of the mechanics of branchial irrigation systems of fishes: Baglioni (2), Babák (1), Leiner (16), Henschel (9, 10), Krogh (15), Black (4), Fry (5), Hughes and Shelton (14), and Hughes (12).

The anatomical mechanism for branchial irrigation in teleosts consists of the mouth and oral cavity, the pharyngeal gills, the branchiostegal apparatus, and the opercula with their enclosed cavities. Specialized muscles and skeletal elements are associated with each of the components. This mechanism moves a continuous or nearly continuous stream of water in the direction from mouth to opercular cavities. Woskoboinikoff (19) and Woskoboinikoff and Balabai (20) described the respiratory flow as being the action of two pumps: the

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F.R.B. No. 663

mouth and oral cavity act as a force pump pushing water over the gills, a the opercula and branchiostegal apparatus act together as a suction pu drawing water over the gills. The gills are arranged in the pharynx in such way as to separate these two kinds of pump by forming a fine screen throu which the water flows on its passage from oral to opercular cavities.

The oral cavity in teleosts is aided in its force pump capacity by one or t oral valves. These valves are classified by Gudger (6) as maxillary (dors or mandibular (ventral). They are velar folds of tissue originating from t mucosa on the inner surface of either or both jaws. These structures a passively and offer no resistance to water entering the mouth as the o cavity enlarges. As the oral cavity is contracted, the valve or valves mo into position closing the opening and preventing regurgitation of wat Thereby, the water inspired is forced over the gills and into the opercul cavities.

Water is drawn from the oral cavity, over the gills and into the opercul cavities, by abduction of the opercula. Entrance of water posteriorly is p vented during this suction phase by the flexible extensions of the posteri ends of the opercula. These maintain contact with the skin covering t lateral margins of the cleithra during abduction of the opercula and functi as valves. Then the opercula are adducted and their valves swing open, allo ing egress of the water which has flowed over the gills.

Woskoboinikoff and Balabai (20) used water manometers to measure t changes in hydrostatic pressure occurring in the oral and opercular cavit during breathing. Later, Hughes and Shelton (13, 14) and Hughes (11, 1 used capacitance manometers together with cinematography to study the ti course of pressure changes in the branchial pump of a number of speci They elucidated the relation between the maxima and minima of hydrosta pressures and the various breathing movements. In so doing, they demonstrat that the pressure in the oral cavity is not always positive with respect to th in the opercular cavities. However, the period during which the opercul pressure exceeds that in the oral cavity is only a small fraction of the respirato cycle. The present study considers this phenomenon with other speci Further, measurements of the amplitude and frequency of pressure chang in the oral and opercular cavities have been made during gentle breathi and various degrees of labored breathing brought on by altering the parti pressure of carbon dioxide in the water.

The Gills

The gills are supported by four pairs of bony arches arranged serially in t pharynx. Each gill arch bears two rows of gill filaments, the hemibranchi and individual filaments bear the secondary lamellae or respiratory plat which provide a large respiratory surface. The tips of adjacent rows of fi ments touch and the lamellae of adjacent filaments within the same r interdigitate with one another leaving small pores for the passage of wat This compact screen forms an advantageous structure for the uptake oxygen. Bijtel (3) has emphasized that the adjacent rows of filament t touch one another during all phases of quiet breathing and that water can p only through the pores between adjacent lamellae.

Studies of Branchial Irrigation Systems

Van Dam (18) and Hazelhoff (8) used fine hypodermic needles inserted behind the opercular valves to extract water which had passed over the gills in order to measure the percentage utilization of oxygen. Similarly, Hughes and Shelton (13, 14) and Hughes (12) used fine hypodermic tubing inserted between the lips and behind the opercular valves to record changes in pressure in the oral and opercular cavities respectively. This technique has two disadvantages. Many fish struggle violently when even a small object is held in the way of the oral or opercular valves. It is therefore difficult or impossible to hold such cannulae in place for long periods in fish not under anesthesia. Moreover, such placement of cannulae interferes with the normal action of these membranous valves.

In the present study van Dam's (18) and Hazelhoff's (8) technique has been modified for measuring hydrostatic pressures in the oral and opercular cavities without interfering with breathing movements. The technique may be used successfully with many species. The observations resulting from the studies employing the modified technique have added to the findings of Hughes and Shelton (13, 14) and Hughes (11, 12) that pressures in the opercular cavity often exceed those in the oral cavity during a small fraction of the respiratory cycle. Moreover, the data arising during the present study made it necessary to determine whether the filament tips of adjacent hemibranchiae remain in contact during moderate and heavy breathing as Bijtel (3) maintains they do during quiet breathing.

Materials and Methods

Species studied were: white sucker, *Catostomus commersoni* (Lacépède); brown bullhead, *Ictalurus nebulosus* (LeSueur); and carp, *Cyprinus carpio* Linnaeus. These were held out of doors in an 850-liter plywood tank which was coated on the inner surfaces with black neoprene to help reduce abrasive injury to the fish. Here the temperature was maintained at 12–16° C in the winter and 15–19° C in the summer. Normal, healthy fish were selected from the stocks and brought inside for thermal acclimation. The temperature was raised to 20° C by daily increments of 1 degree. All fish used in this investigation were acclimated at 20° C, the minimum period of acclimation being 4 weeks.

USE OF CANNULAE

Cannulae were used together with segment tambours to measure changes occurring in the oral and opercular cavities during breathing. The difficulties encountered when cannulae are introduced between the lips into the oral cavity and behind the opercular valve into the opercular cavity have been indicated in the Introduction. A new technique was developed during the present study whereby these difficulties were overcome. Cannulae were inserted in the oral and opercular cavities through the tissues adjoining these structures.

Cannula Material

The material used for cannulae was Intramedic Polyethylene Tubing produced by Clay-Adams Inc., New York. This tubing is ideal for such use

because it is pliable even when cold and does not cause tissue irritation. The size used was PE 160 with an outside diameter of 0.152 mm and an inside diameter of 0.114 mm.

Operative Procedures

Specimens were removed from the acclimation tanks before feeding time on the day preceding an experiment. They were anesthetized in a 1/20,000 solution of MS 222 (tricaine methanesulphonate). The fish were immobilized in less than 3 minutes and they resumed breathing and swimming movements within 2 minutes after they had been returned to fresh water.

The Cannula for the Opercular Cavity

A small horizontal hole was made through the cleithrum by means of a No. 18 hypodermic needle which is of approximately the same outside diameter as that of the cannula to be inserted. A length of polyethylene tubing was cut obliquely to provide a sharp point to facilitate its passage through the hole in the cleithrum. Several inches of tubing were drawn through the hole while great care was taken to avoid touching the gills. The cannula was bent clear of the gills and operculum, and the pointed end cut off. A small flange was made on the end of the tube by holding it near a small flame. When the flange was pulled tightly against the posterior wall of the opercular cavity, it provided a tightly fitting placement of the cannula. This operation was easily completed in 1 minute. The placement of the cannula in the cleithra of the species studied is shown in Fig. 1.

The Cannula for the Oral Cavity

It was difficult to find a suitable location for the cannula for the oral cavity. The lips, sides, and floor of the mouth are constantly being moved as the fish breathes. This precluded placing the cannula in any of these locations. An ideal place was found to be the ethmoid region of the rostrum on the midline just anterior to the nostrils. A small hole was made vertically through the rostrum to the roof of the mouth and tubing was introduced as in the opercular cavity. A small plastic washer was put on the cannula before the end was flanged. This prevented the cannula from being pulled out. Figure 2 shows the cannula for the oral cavity in a bullhead skull.

The location of the cannula varied slightly in the three species. In suckers and carp it penetrated the space anterior to the unpaired dermethmoid and posterior to the paired maxillary bones. In the bullhead the cannula entered

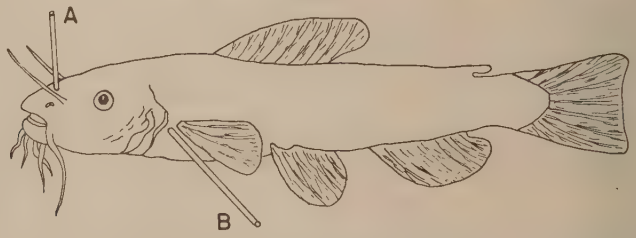

FIG. 3. Sketch of bullhead showing two cannulae in place: A, cannula to the oral cavity; B, cannula to the opercular cavity.

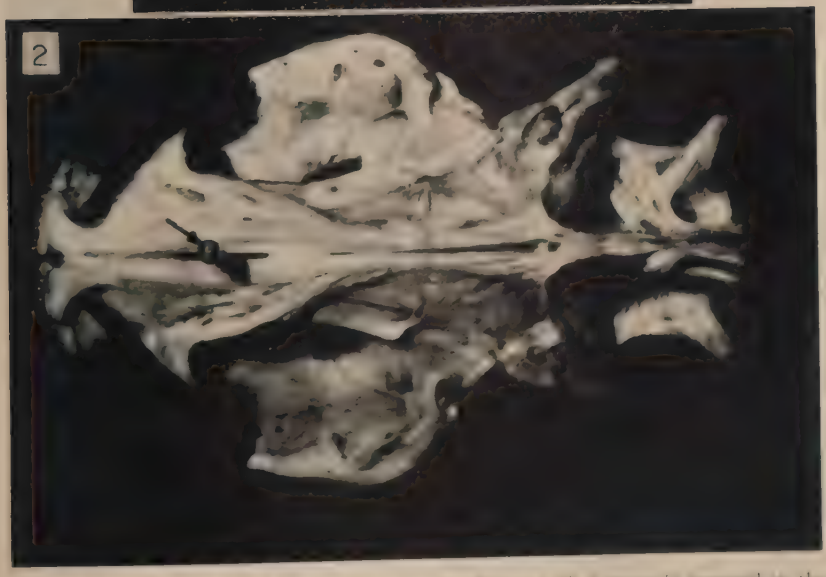

FIG. 1. Paired sagittae of bullhead, sucker, and carp with a centimeter inserted in the left cavity of each pair.

FIG. 2. Skull of bullhead with centimeter rod and needle inserted through the rostrum.

Photographed by J. Ash.

the foramen between the paired frontals and the unpaired dermethmoid and emerged ventrally through the vomer just anterior to the parasphenoid. The breathing movements were not impeded by the cannulae fixed in these locations. Moreover, the cannulae passed well anterior to the brain and in a region believed free of cranial nerves and principal blood vessels. Figure 3 shows the two kinds of cannulae in position in a bullhead.

Following the operative procedures described above, the fish were placed in running tap water at 20° C. Here they could recover from the operation and the anesthetic. The fish were invariably resting and breathing quietly when observed next morning. The cannulae were removed after completion of experiments and the fish were returned to acclimation tanks. In most cases the small holes left after removal of the cannulae healed quickly and the fish could be used again in about 2 months.

MEASUREMENT OF CHANGES IN PRESSURE IN THE ORAL AND OPERCULAR CAVITIES

Apparatus

Direct measurements were made of changes in hydrostatic pressure in the oral and opercular cavities of fish confined in a perforated, plexiglass tube 3 in. in diameter. This tube rested on the bottom of an aquarium of 23-liter capacity which was supplied with running water in which the oxygen and carbon dioxide levels could be varied. The cannulae, leading from the oral and opercular cavities were attached by means of drawn-glass connections to quarter-inch Tygon tubing. The Tygon tubing was in turn connected to a glass tube flared slightly at the distal end and held within a glass water jacket like that shown in Fig. 4. The water jacket has two projecting arms, a horizontal one for connection with the aquarium and a vertical one for a water levelling tube and bubble trap. A piece of thin latex rubber was stretched loosely over the flared end of the glass tube and cemented in place. This operation must be done so that the resulting diaphragm has no tension, yet is without slack. This diaphragm serves as a segment tambour, which must be very sensitive to changes in hydrostatic pressure. The care exercised in attaching the latex

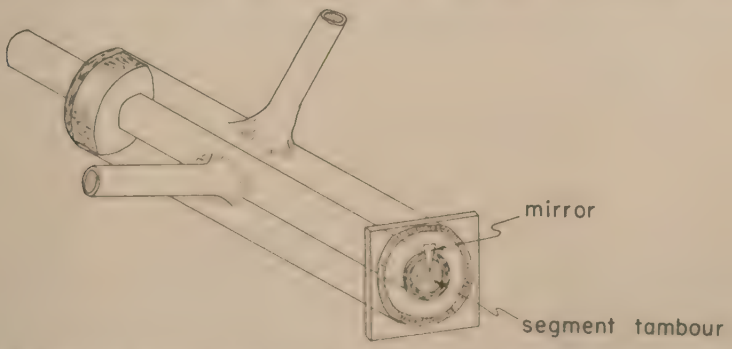

FIG. 4. Segment tambour held within water jacket. The segment tambour is separated from the flat end of the water jacket by a distance of about 1/8 of an inch. This permits unrestricted movement of the segment tambour and the mirror.

rubber ensures sensitivity to hydrostatic pressure changes, especially in the range near zero pressure. A small, triangular piece of mirrored glass, made from an aluminum-coated cover slip, was then cemented to the segment tambour. The segment tambour was held in the water jacket, which was connected to the aquarium to keep the same hydrostatic pressure acting on the outer surface of the rubber membrane as was acting on the fish in the aquarium. (The segment tambour is shown in Fig. 4.)

The connections including cannulae, glass, and Tygon tubing were kept as short as possible to reduce resistance to fluid motion. The connections from the oral and opercular cavities to their respective segment tambours were made the same length to equalize the surface resistance. The resulting system was an extremely sensitive indicator of changes in hydrostatic pressure within the oral and opercular cavities. An observer could easily count the fish's breathing movements by watching the segment tambours.

The recording system was completed by an optical lever. This pressure recording system is shown diagrammatically in Fig. 5. Separate projection lamps (10, in Fig. 5) were focused on each segment tambour (9, in Fig. 5) and the light reflected from the mirrors on the segment tambours was directed onto a plano-convex lens mounted a fixed distance in front of a variable-

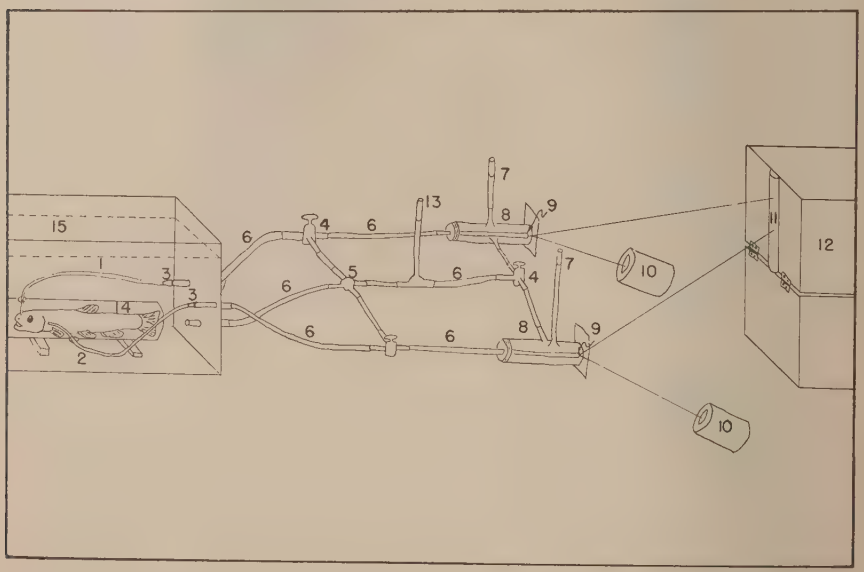

FIG. 5. Diagram of the apparatus used for recording the time course of changes in pressure in the oral and opercular cavities: 1, cannula from oral cavity; 2, cannula from opercular cavity; 3, drawn-glass adapters for connecting cannulae to larger-sized tubing; 4, three-way glass stopcocks for aligning segment tambours with the fish's branchial pump system or with the aquarium; 5, four-way glass connection; 6, Tygon tubing; 7, levelling tubes, bubble traps; 8, glass water jackets; 9, segment tambours; 10, light sources; 11, plano-convex lens; 12, hinged box containing revolving drum; 13, bubble trap; 14, perforated chamber for holding fish; 15, aquarium.

speed revolving drum. This lens was made by sawing a piece of 1-in.-diameter plexiglass rod along the median longitudinal plane. The rough surfaces were smoothed with sandpaper and the whole lens polished with jewelers' rouge and a buffing wheel. The beam of light projected on the segment tambour was made a thin, horizontal strip by passing it through a slit between two razor blades held within a frame which was mounted in front of the light source. Two strips of black tape were attached to the plane surface of the plexiglass lens leaving a narrow slit between them. The reflected light from the mirror was a thin, horizontal strip. This strip was reduced to a small point of light on the revolving drum after it passed through the vertical slit between the strips of tape.

Permanent records of pressure changes during the breathing cycle were made on Kodagraph extra thin, fast projection paper mounted on the revolving drum. The latter was geared for precise speeds of rotation of 1, 5, and 15 seconds as well as 1 and 5 minutes.

Recording Changes in Pressure

A standard procedure was adopted for recording changes in hydrostatic pressure associated with breathing. The room was darkened and the projection lamps turned on for prescribed periods after the segment tambours for the oral and opercular cavities had been connected with these cavities or with the aquarium. The segment tambours were made to register zero pressure by closing the three-way stopcocks (4, in Fig. 5) to the cannulae and opening them to the aquarium. The zero reference pressure was recorded for each segment tambour during one rotation of the drum. These reference marks were horizontal, parallel straight lines. Then the three-way stopcocks were opened to the cannulae and some breathing movements recorded with the drum stopped. The resulting records were two parallel, vertical straight lines which showed only the amplitude of the changes in pressure during the breathing cycle. The beam of light from each mirror entered the lens at a different

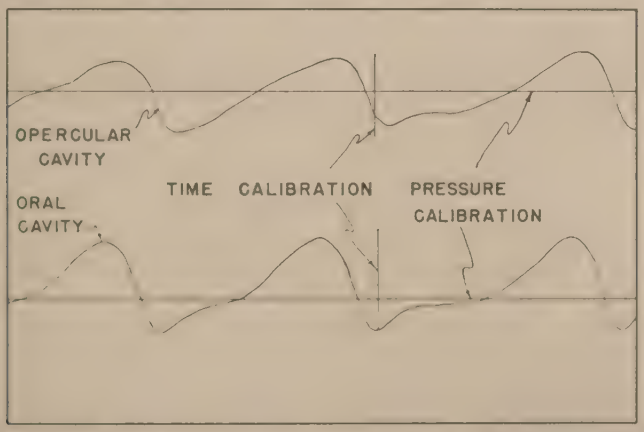

FIG. 6. Tracings from a photographic record showing time course of changes in hydrostatic pressure in the oral and opercular cavities of a white sucker. Note respective calibration lines for time and pressure.

angle with the result that the vertical lines on the record were a certain distance apart. The intersection of each vertical line with its respective horizontal line gave a point of reference which represented zero pressure and time. Finally, a number of breathing cycles were recorded with the drum rotating. A set of curves illustrating the changes in hydrostatic pressure during the breathing cycle of the sucker is given in Fig. 6.

Calibrating Segment Tambours

Since no two segment tambours had the same elasticity, the resulting pressure records made simultaneously with different tambours were not directly comparable. This difficulty was overcome by calibrating each segment tambour and then correcting one to the other. A U-shaped water manometer was attached to the levelling tube (7, in Fig. 5) by means of rubber tubing. The stopcocks were closed. A piece of paper ruled in millimeters from zero to plus and minus 25 was stuck over the vertical lens and the beam of light made to rest at zero. Then the light beam was made to come to rest at a number of places above and below the zero mark. This was done by applying pressure or suction to the open end of the U-shaped manometer. The pressure in cm of water and the corresponding deflection of the light beam in mm measured on the ruled paper were recorded for a number of deflections above and below zero deflection. The results were plotted as deflection in mm against pressure in cm of water. This procedure was repeated for each segment tambour on each day it was used.

The information gathered in this way made it possible to correct readings using one segment tambour to those with another. For example, if one tambour gave a deflection of 7.5 mm for a pressure of 10 cm of water and a second one gave a deflection of 6.2 mm for the same pressure, then the first is more sensitive than the second by a factor of 1.21. The readings from the second tambour can be made comparable with those of the first by multiplying them by 1.21. One set of curves can be made to have the same relation of deflection to pressure as another by measuring the distances from zero pressure to the curve at regular intervals along the record, multiplying by an appropriate factor, and plotting the new points. This is a very slow and tedious process. A simple drafting machine, with which this process can be done more quickly and easily, was constructed by Dr. F. E. J. Fry. It is a modification of the pantograph.

OBSERVATIONS OF THE OPENING OF GILL SLITS

Movements of the gill filaments and the opening and closing of the gill slits were observed in the three species under consideration here. The opercular valves typically open after completion of opercular abduction and remain open for the first one-third to two-thirds of the adduction phase. The gills, thus briefly exposed, are seen to move, and transitory openings appear between adjacent hemibranchiae of neighboring gills. Since the openings to the opercular chambers are very small and the gill slits remain open only briefly, this opening was recorded using cinematography with high shutter speeds. Cameras (8-mm and 16-mm) were hand-held at close range to record the opening of the gill slits.

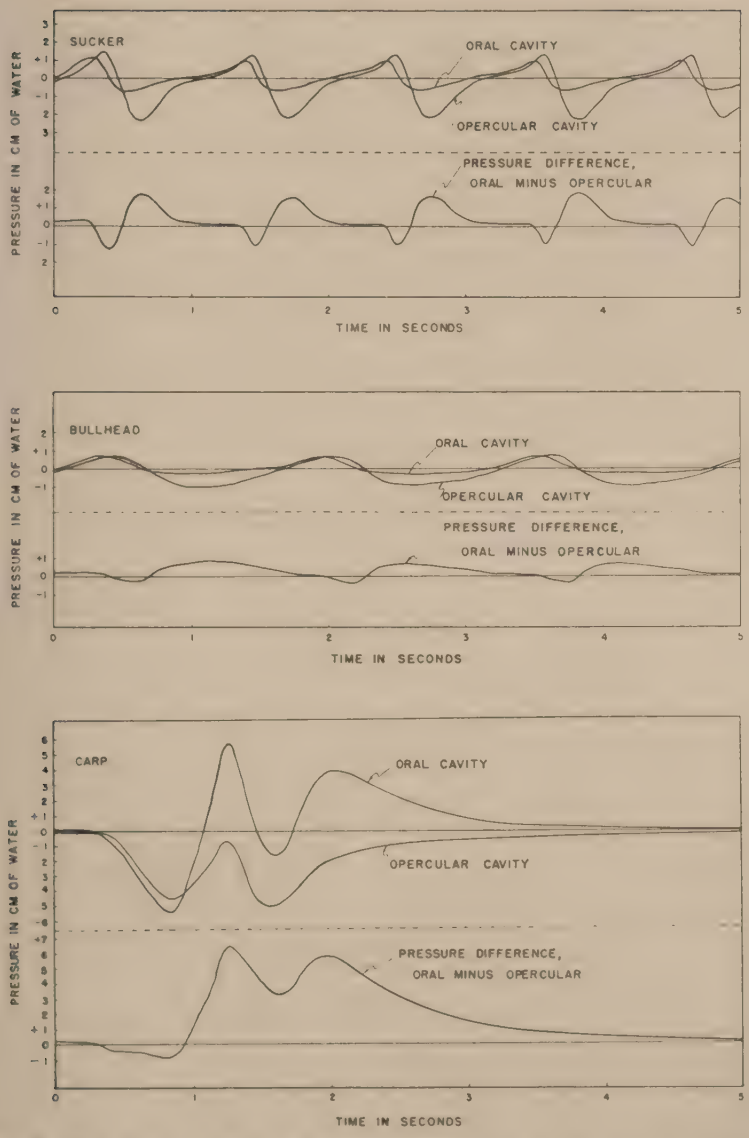

FIG. 7. Time course of changes in hydrostatic pressure in the oral and opercular cavities of sucker, bullhead, and carp during quiet breathing. These pressure curves have been reproduced from the original photographic records by the method described in the text. The curves presenting the differences in pressure between the oral and opercular cavities were obtained by measuring the vertical distances at regular intervals between the curves for pressures in these cavities and plotting the values on separate ordinate scales in the lower part of each drawing.

Results

HYDROSTATIC PRESSURES IN THE BRANCHIAL PUMP

Quiet Breathing

The time course of changes in hydrostatic pressure in the oral and opercular cavities was measured and recorded for a number of quietly breathing suckers, bullheads, and carp. These temporal changes in hydrostatic pressure are illustrated in Fig. 7, which shows some typical respiratory patterns for quietly breathing fish. These records of hydrostatic pressure in the oral and opercular cavities are plotted concurrently to show their rhythmic nature and their relations one to another. Pressures in the oral cavity were predominantly positive with respect to those in the opercular cavities. This relation between the pressures in the two cavities must exist if water is to flow from oral to opercular cavities.

One salient feature common to all three breathing patterns was a brief phase during each breathing cycle when the pressure in the opercular cavity exceeded that in the oral cavity. Such phases of back-pressure are illustrated in Fig. 7 by the curves relating differences in pressure, which were obtained by subtracting the hydrostatic pressure in the opercular cavity from that in the oral cavity. These values are plotted on separate ordinate scales below each set of pressure records. The back-pressures, if maintained, would result in a back-flow of water over the gills, but, as shown in Fig. 7, they occupy only small portions of the entire breathing cycles in bullheads and carp and a slightly larger portion of the breathing cycle of the sucker. Hughes and Shelton (14) found a similar back-pressure phase of the breathing cycles in their studies of the time course of pressure changes in brown trout (*Salmo trutta*), tench (*Tinca tinca*), and roach (*Leuciscus rutilus*). Hughes (12) extended these studies to marine fishes and found back-pressures in the breathing cycles of the following species: herring (*Clupea harengus*), whiting (*Gadus merlangus*), horse mackerel (*Trachurus trachurus*), wrasse (*Crenilabrus melops*), and five-bearded rockling (*Onos mustela*), but not in the bottom-living fishes: butterfly blenny (*Blennius ocellaris*), plaice (*Pleuronectes platessa*), and merry sole (*Microstomus kitt*).

Although there is an intermittent intake of water by the mouth and an intermittent discharge from the opercular cavities, the pressure difference curves show that the flow of water over the gills is nearly continuous. It is possible by inspection of the pressure records and their respective pressure difference curves to evaluate the relative contributions during gill irrigation of the opercular cavities as a suction pump and the oral cavity as a pressure pump. The action of the suction pump is predominant and that of the pressure pump is only of minor importance in gill irrigation of the sucker and bullhead. This is clearly shown in the records, where the maximum excursions of the pressure difference curves correspond with the maximum negative pressures recorded in the opercular cavities. Similarly, the oral cavity makes its greatest contribution as a pressure pump when the maximum excursions of the pressure difference curves correspond with the maximum positive pressures recorded in the oral cavity. Such predominance of the oral pressure pump was observed in the carp, where it contributed slightly more to gill irrigation than did the suction pump.

Outstanding differences were found in the rate of breathing and the amplitude of changes in hydrostatic pressure in the respiratory cycles of bullheads, suckers, and carp. The breathing rates of the fish described in Fig. 7 were approximately 65, 38, and 24 for sucker, bullhead, and carp respectively. These species all displayed periods of apnea when resting quietly in air-saturated water at 20° C. Bullheads occasionally suspended breathing for as long as 30 seconds; suckers rarely suspended breathing for 10–15 seconds. While resting bullheads and suckers breathe regularly with only occasional periods of apnea, carp breathing is characterized by trains of two or three rapid movements followed by an apneic period. One train of two to three respiratory movements and the accompanying apneic period lasted just 5 seconds in the example given. This alternation between breathing and apneic periods is repeated rhythmically and has been recorded continually for as long as 5 minutes in resting carp. The effect of this peculiar pattern of breathing in the carp is to build up a considerable positive pressure on the oral side of the gills. This pressure is increased during about $1\frac{3}{4}$ seconds and is gradually attenuated during the succeeding $3\frac{1}{4}$ seconds when the breathing rate is 24 per minute.

There were striking differences among the three species in the amplitude of pressure changes during breathing of individuals of equal size. The amplitude of the sucker's pressure excursions is over twice that of the bullhead, while the amplitude of breathing pressures in the carp is over twice that of the sucker.

Heavy Breathing

Heavy breathing was induced by raising the partial pressure of free carbon dioxide. The observable effects of these high levels of carbon dioxide were increases in the rate and depth of breathing. This is illustrated in Fig. 8. The breathing rates at the times these records were made were approximately 120, 50, and 110 in sucker, bullhead, and carp respectively. In all three species the rates were more regular than those usually observed during quiet breathing. There were few pauses or changes in amplitude, both of which were frequently recorded during quiet breathing. The most striking change from quiet to heavy breathing was that displayed by the carp. The slow, intermittent pattern of breathing was replaced by a rapid, regular one.

The general relations between the hydrostatic pressures in the oral and opercular cavities of the sucker and bullhead are only slightly changed from those observed in quietly breathing fish. The capacity of the opercular cavities to operate as a suction pump still appears to have the dominant action in irrigating the gills. But the comparative contribution of the pressure pump of the oral cavity is greater during this heavy breathing than during quiet breathing. The breathing patterns of heavily breathing suckers and bullheads retain the brief back-pressure phases. However, the back-pressure phases in these suckers and bullheads are of shorter duration and are lower in magnitude than those recorded during quiet breathing.

The breathing patterns of both quietly and heavily breathing bullheads and suckers differ only in small details from those recorded by Hughes and Shelton (14) for trout, tench, and roach. There is a remarkable similarity

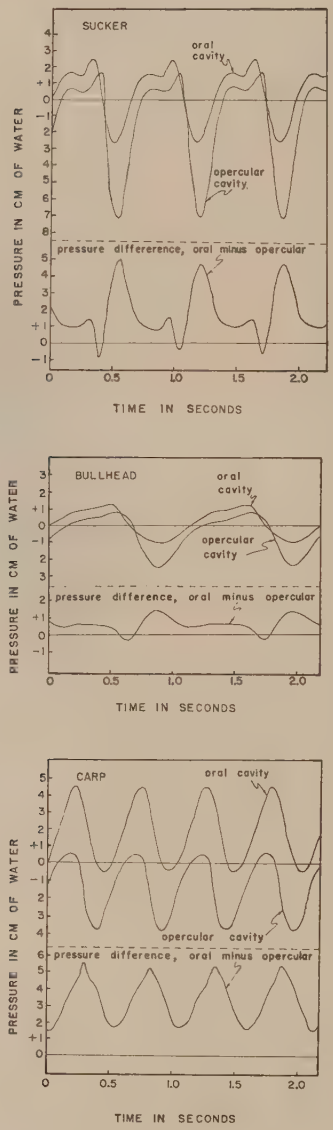

FIG. 8. Time course of changes in hydrostatic pressure in the oral and opercular cavities of sucker, bullhead, and carp during heavy breathing. The fish were induced to breathe heavily by increasing the ambient carbon dioxide level. These pressure curves were reproduced from the original photographic records by the method described in the text. The curves relating differences in pressure were obtained by measuring the vertical distance at regular intervals between the curves for pressures in the oral and opercular cavities and plotting the values on separate ordinate scales.

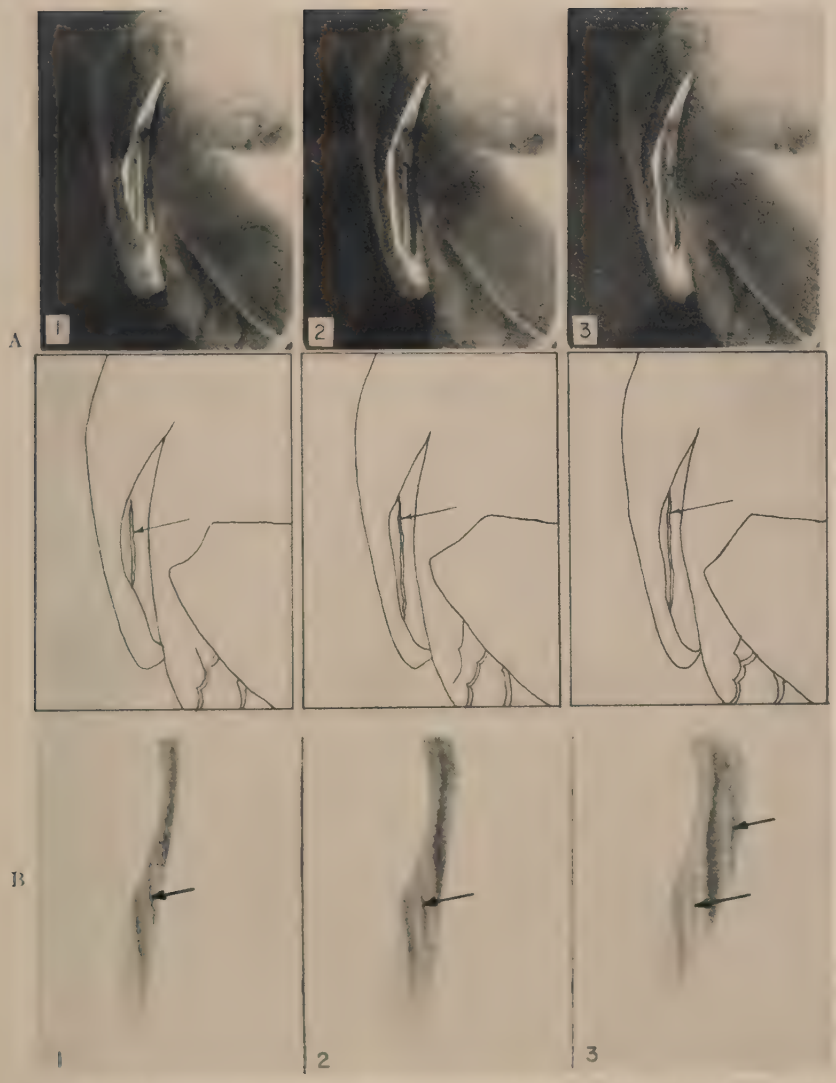

FIG. 9. Ciné photographs showing an open gill slit in a carp (A) and in a sucker (B). Arrows indicate gill slits.

A. These are negative prints enlarged from a black and white, 8-mm ciné film (positive). This sequence shows the opercular valve opening (1 and 2) and fully open (3). In each of these photographs the opening between the second and third gills appears as a white slit. The accompanying drawings are given to point out the opened gill slit in each photograph.

B. These are positive prints enlarged from a 16 mm black and white ciné film. This sequence shows the progressive opening of the opercular valve (1–3) and of two gill slits within the opercular cavity.

between Hughes' and Shelton's records of the breathing pattern in the brown trout and that of the heavily breathing sucker in the present study.

The respiratory pattern of the heavily breathing carp differs greatly from those of suckers and bullheads. There is also little similarity between the pressure curves for the carp in the present study and those of the tench and roach as recorded by Hughes and Shelton (14). All three of these latter species belong to the family Cyprinidae. In heavily breathing carp the pressure in the oral cavity remains higher than that in the opercular cavity during all phases of the respiratory cycle. Hughes (12) has recently reported a similar finding for certain marine flatfishes. Consequently, the breathing patterns of the carp and certain marine flatfishes have no back-pressure phases and therefore no reversal of flow of water over the gills.

Results of the present study indicate that the pressure relationships between the oral and opercular cavities of the branchial pump system are such that water will flow in the direction from oral to opercular cavities during most of the breathing cycle. Therefore, the gills are bathed by a continuous or nearly continuous stream of fresh water. Van Dam (18) concluded that ". . . *the water current along the gills of the rainbow trout must be continuous.* I think it very probable that the same applies to most, if not all, fish species." The present findings concerning breathing patterns in suckers, bullheads, and carp are in general agreement with van Dam's momentous conclusions.

OBSERVATIONS OF RHYTHMIC OPENING OF THE GILL SLITS

Numerous observations were made of the movements of the gills during moderate and heavy breathing. This was done by peering into the opercular cavity during the brief phase of the breathing cycle when the opercular valve is open. It was observed in moderately and heavily breathing suckers, bullheads, and carp that openings appear consistently between the hemibranchiae of adjacent gill arches during the period when the opercular valves are open. No such observations were possible when the fish were breathing quietly because the opercular valves do not open sufficiently.

A series of ciné photographs was made showing the opening of the gill slits in suckers, bullheads, and carp. Several sequences from the films showed very clearly the opening of the gill slits when the films were projected on a screen. A sequence of photographs of the head and gills of a carp and of the gills only of a sucker is shown in Fig. 9 together with drawings on which some of the details are indicated. These photographs show the open gill slits.

Discussion

OPENING OF THE GILL SLITS

An important aspect of fish breathing which has been overlooked by previous workers is the rhythmic opening of the gill slits during moderate and heavy branchial irrigation. Much of the water bathing the gills flows from the oral to the opercular cavities not through the tiny interlamellar spaces but via the openings which appear periodically between the hemibranchiae of adjacent gills. The present author has observed this phenomenon, the opening of the gill slits, in several species and believes it to have a significant effect in the operation of the gills.

Several authors (Woskoboinikoff (19), van Dam (18), Krogh (15), Bijtel (3), Hughes and Shelton (14), and Nicol (17)) have accepted as fact that the tips of filaments of adjacent hemibranchiae remain in contact throughout the normal breathing cycle and that water can pass only through the spaces between adjacent lamellae. Bijtel (3) reported, "As the gill-cover is raised for a moment in every expiration, it is possible to watch the position of the filament tips by looking under the gill-cover in this phase of breathing. Mrs. Hofdijk-Enklaar found, that in this phase of the respiration the filament tips of one hemibranchia and those of the successive one of a following branchial arch lean against each other. So during quiet respiration the aperture between two successive gills is closed in expiration." This is quite likely true during quiet breathing, when little water flows over the gills. But this idea must be rejected when heavy breathing is considered. Rhythmic opening of the gill slits was seen during the present study in most instances when the fish were breathing heavily enough for the opercular opening to widen sufficiently for an observer to see the gills. The present author was unable to observe the gills of quietly breathing fish because the opercular opening did not widen sufficiently to expose the gills.

Hughes and Shelton (14) accepted Bijtel's opinion that the gill slits remain closed during quiet respiration and tacitly accepted that this applies as well to moderate and heavy breathing. With the acceptance that the gill slits remain closed during heavy breathing, they discussed the difference in pressure which exists between the oral and opercular cavities and attempted to evaluate this in terms of gill resistance. By an experiment in which they caused a tench to vary the rate of flow of water over the gills by changing the $p(CO_2)$, they showed that the ratio of the mean pressure difference across the gills to the minute volume decreases as the minute volume and mean pressure difference increases. Hughes and Shelton reported, "It is clear, then, that under these conditions the gill resistance is variable and that it probably decreases as the pressure difference across the gills increases." They cited, as further evidence for the above conclusion, that the ratio of the mean pressure difference to the minute volume decreased as water was made to flow over the gills of a deeply anesthetized, non-breathing fish.

Observations arising during the present study permit a simple explanation for the phenomenon observed by Hughes and Shelton. Gill resistance must indeed decrease with increased opening of the gill slits. The present author reasons that the gill slits may remain closed during quiet breathing when little water flows over the gills. At higher rates of flow of water, the gill slits are either forced open by the rush of water through the pharynx or are opened actively during the breathing movements which result in flexure and contraction of the gill arches. The result of greater and greater opening of the gill slits would be a diminishing gill resistance because the water would flow through the rather large gill slits rather than through the restricted passages between adjacent lamellae.

A further consequence of opening of the gill slits is a reduction in the difference in pressure between the oral and opercular cavities and a slowing of the rush of water over the gills. This reduction in the difference in pressure between

either side of the gills could serve as an effective safety device protecting the delicate gill membranes from increased hydrostatic pressures resulting from heavy breathing. Since the difference in pressure between the oral and opercular cavities may be increased considerably in passing from quiet to heavy breathing, it is conceivable that heavy breathing could result in damage to the delicate lamellae or restriction of the flow of blood through them were there not some measure for their protection.

Opening of the gill slits could assume its greatest importance during swimming. Presumably, great volumes of water can easily be passed over the gills as a result of the fish's forward movement through the water. Although the species considered in the present investigation do not depend entirely on forward motion through the water for branchial irrigation, the mackerel does and will be asphyxiated if prevented from swimming (Hall (7)). Were the gill slits to remain closed, all the water would have to pass through the interlamellar spaces with the consequence of possible damage to the delicate lamellae. Moreover, were there no means of easing the passage of water through the gills of rapidly swimming fish, it is conceivable that there would be a considerable drag placed on the fish, thus offsetting any effects of body streamlining in overcoming resistance to motion through the water.

The continuity and magnitude of the gradient in hydrostatic pressure across the gills are important considerations in the study of the efficiency of branchial irrigation. The back-pressure phase of the breathing cycle, when water flows from opercular cavities to the oral cavity, must be detrimental to efficiency of the uptake of oxygen. However, this phase is of short duration in relation to the whole breathing cycle and is probably of minor importance. The continuity and magnitude of the positive pressure gradient in the oral cavity is illustrated in Figs. 7 and 8. Within a single respiratory cycle, the magnitude of the pressure gradient varies considerably. This would seem *a priori* to result in changing degrees of efficiency of gaseous exchange from one moment to the next during the respiratory cycle. The pressure difference curves in Figs. 7 and 8 are indicative of the volume of water passing through the interlamellar spaces. The species which maintains the most constant positive pressure gradient over the gills would appear to be the best able to take up oxygen from the water. The afore-mentioned phenomenon of opening of the gill slits could modulate the differences in hydrostatic pressure, at their upper levels, between either side of the gills in such a way as to function as a safety valve preventing too great differences in hydrostatic pressure between the oral and opercular cavities. Such a mechanism could operate to regulate the upper limit of velocity of water through the interlamellar spaces.

Summary

White suckers (*Catostomus commersoni*), brown bullheads (*Ictalurus nebulosus*), and carp (*Cyprinus carpio*) were used in these studies of the irrigation of the gills. Measurements were made of the changes in hydrostatic pressure in the branchial pump during various intensities of breathing by means of two cannulae of polyethylene tubing having a small diameter. The ends of these cannulae were fixed in the oral and opercular cavities respectively and they

were connected with segment tambours having small mirrors mounted on them. Photographic records were made of changes in hydrostatic pressure in the oral and opercular cavities by using an optical lever system.

1. The observations of the relationships of hydrostatic pressure within the branchial pump are in general agreement with those of Hughes and Shelton (13, 14) and Hughes (12), who found in other species that pressures in the oral cavity are positive with respect to those in the opercular cavities for all but a brief phase of the respiratory cycle when there is a back-pressure acting to reverse the flow of water over the gills.

2. The carp has a peculiar breathing pattern. In quietly breathing individuals the pattern consists of two or three rapid movements followed by a period of apnea. The whole cycle of breathing movements and apneic period often lasts about 5 seconds.

3. The slits between adjacent hemibranchiae of neighboring gills open rhythmically during moderate and heavy breathing. This allows some of the water to bypass the interlamellar spaces, which a number of previous workers insist provide the only course of water through the gills. The suggestion is given that opening of the gill slits is not entirely detrimental but may act as an effective safety device protecting the delicate gills from excessive hydrostatic pressures.

Acknowledgments

I am grateful to Professor F. E. J. Fry under whose enthusiastic direction this work was done. I appreciate the advice given during the writing of the manuscript by Professor K. C. Fisher and other members of the staff of the Department of Zoology.

Doctors Ian McCausland and B. S. Muir, and Messrs. E. T. Cox and J. Sweet, helped with the photography.

I am grateful to Mr. D. Crosby for his help in the laboratory and to Mr. R. B. Jones who helped care for the fish.

Financial support was given by the National Research Council, the Ontario Research Council, and the Ontario Research Foundation and made available through the Ontario Fisheries Research Laboratory.

I appreciate the many services given by the personnel of the Ontario Department of Lands and Forests who are associated with the Laboratory for Experimental Limnology, Maple, Ontario, where this work was done.

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ISOLATION AND IDENTIFICATION OF ADRENOSTERONE IN SALMON (ONCORHYNCHUS NERKA) PLASMA

D. R. Idler, P. J. Schmidt, and I. Bitners

Adrenosterone, in a crystalline form, has been isolated from extracts of human adrenal cortex (1). It has also been identified in the perfusate obtained when blood containing added ACTH was circulated through bovine glands (2). To our knowledge the presence of adrenosterone in peripheral plasma has not previously been reported.

In a study involving the quantitative estimation of various steroid hormones in sockeye salmon plasma (3), an unidentified ultraviolet (U.V.) absorbing spot was detected when plasma extracts prepared from postspawned male sockeye were chromatographed on paper with a heptane : 80% methanol solvent system for 16 hours. The polarity of the new substance was identical with that of adrenosterone and intermediate between 17α-hydroxyprogesterone and its 20β-dihydro-epimer, both previously isolated from salmon plasma. The Zimmermann reagent produced the violet color, typical of 17-ketosteroids, with the new steroid.

Sockeye salmon plasma was obtained from postspawned fish taken at Cultus Lake in November, 1960. The procedure for bleeding the fish and for extracting the plasma, and the chromatographic methods used, have been described elsewhere (3).

The extract from 500 ml of plasma was partitioned between 70% methanol and hexane (3). The 70% methanol fraction was then taken to dryness and chromatographed on paper using the heptane : 80% methanol system for 16 hours. The reference standard of adrenosterone and the corresponding spot on the plasma strip were detected by U.V. light (4) and had identical mobilities ($R_T = 0.72$ cm hr). The plasma spot was eluted with methanol using descending chromatography. By U.V. absorption at 240 mμ (corrected) (3), and based on the recovery of adrenosterone through the procedures, there was 2.5 μg 100 ml of the steroid in the plasma. The plasma was eluted from the paper, evaporated to dryness, and subjected to sodium borohydride reduction (5). The reduction product and 11-ketotestosterone were chromatographed on paper using heptane:benzene (1:1) — 70% methanol for 3 hours. The plasma spot and the reference standard were detected with U.V. light and both had identical mobilities.

The substance from the plasma was eluted from the paper, treated with 0.1 ml of acetylating mixture, and left at room temperature for 24 hours. The reaction mixture was taken to dryness using N_2 at 40°. The acetylating mixture consisted of 5 volumes of pyridine, 4 volumes of benzene, and 1 volume of acetic anhydride. The acetylated product was chromatographed along with 11-ketotestosterone acetate using the heptane : 80% methanol solvent system for 3.75

hours and the mobilities of the steroids were identical (R_T = 4.1 cm/hr).

Acknowledgment

Staff of the International Pacific Salmon Fisheries Commission rendered valuable assistance in obtaining the fish.

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Received June 8, 1961.
Fisheries Research Board of Canada,
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Vancouver 8, B.C.

TESTOSTERONE, CONJUGATED AND "FREE", IN THE BLOOD OF SPAWNED FRASER RIVER SOCKEYE SALMON (ONCORHYNCHUS NERKA)[1]

DOV GRAJCER AND D. R. IDLER

Abstract

Testosterone was isolated from both the "free" and conjugated steroid fractions obtained from the plasma of spawned female sockeye salmon. The structure of the steroid was confirmed by several criteria including a sulphuric acid chromogen and infrared spectra. Evidence is presented for the presence of testosterone in the conjugated steroid fraction prepared from spawned male sockeye. Several unsuccessful attempts were made to detect dehydroepiandrosterone and androsterone.

Introduction

Cortisol, cortisone, corticosterone, 17α-hydroxyprogesterone and its 20β-dihydroepimer, and 11-ketotestosterone have recently been isolated from the "free" steroid fraction obtained from the plasma of sockeye salmon (1, 2, 3). Reichstein's substance "S" has been tentatively established to be present while the evidence for aldosterone is inconclusive (1, 2, 4).

To our knowledge, no study has yet been reported concerning the isolation of conjugated steroids from fish blood. This investigation had its beginning in an attempt to establish the presence, in salmon plasma, of dehydroepiandrosterone and androsterone, the principal steroids in normal human plasma. These efforts led to the discovery of a conjugated steroid with a Δ^4-3-ketone moiety and this substance has been identified as testosterone.

Methods

Plasma

Blood was obtained from spawned Chilko Lake male and female sockeye salmon in September, 1959, spawned Cultus Lake females on November 28, 1960, and Siwash Bridge females on August 28, 1959. Fish were bled by severing the caudal artery and precautions were taken to exclude slime and excreta. The heparinized blood was chilled on ice, centrifuged promptly at 4000–5000 r.p.m., and the plasma stored in polyethylene containers on dry ice and later at −35° C. The "free" steroids were extracted from the alkalized plasma three times with 2.5 volumes of dichloromethane or ethyl acetate as previously described (3).

Isolation of Conjugated Steroids

Glucuronides.—The plasma residue from the extraction of the free steroids was treated with 2.5 volumes of ethanol to precipitate the proteins and the precipitate was removed by centrifugation at 9000 *g*. The precipitate was

[1]Manuscript received April 27, 1961.
Contribution from the Fisheries Research Board of Canada, Chemistry Section of the Technological Station, Vancouver 8, B.C.

washed with 70% ethanol and the washings were added to the supernate. The entire procedure was carried out at 0° C. The supernate was evaporated *in vacuo* at ca. 30° C using a flash evaporator, the receiver of which was cooled in a dry ice – acetone mixture. The residue was suspended in a volume of water equivalent to that of the plasma and extracted three times with dichloromethane to remove any steroids which might have been released from the protein. Dichloromethane was used as a preservative. Versene (5 mg/100 ml) was added and the aqueous phase was adjusted to pH 4.5 and treated with β-glucuronidase (110 mg/100 ml),* at 37° C for 48 hours. The pH was then adjusted to 3.5 and the hydrolysis was continued for an additional 24 hours. Prior to use the β-glucuronidase was suspended in water and extracted three times with 2 volumes of dichloromethane. Steroids obtained by this procedure will be referred to as glucuronide conjugated ("G"). The steroids freed by the glucuronidase treatment were removed by three extractions with dichloromethane. The dichloromethane extract was washed three times with 0.1 volume of each of the following: 5% sodium bicarbonate, 0.1 *N* acetic acid, and water. The organic solvent was removed *in vacuo* as described above and the residue was partitioned between 70% methanol and hexane. The methanol was removed by flash evaporation, the residue taken up in water, and the "G" steroids extracted with dichloromethane.

Sulphate conjugates.—The aqueous phase from the extraction of the "G" steroids was made 2 *N* with respect to H_2SO_4 and 1 volume of ethyl acetate was added (5). The two phases were thoroughly equilibrated in a separatory funnel for several minutes and the ethyl acetate phase incubated at 37° C for 48 hours. The ethyl acetate was washed with sodium bicarbonate, 0.1 *N* acetic acid, and water. This fraction will be referred to as the sulphate conjugate (SO_4).

Residual conjugates.—The H_2SO_4 phase from the liberation of the SO_4 conjugates was neutralized with concentrated NaOH to a pH of 0.8 and the solution was continuously extracted with ether for 48 hours. A small amount of water was added to the ether (to prevent a high acid concentration when the volume of the solution was reduced) and the ether was removed at room temperature using a flash evaporator as described above. More water was added to the aqueous suspension of the residue which was then extracted with ethyl acetate and washed with sodium bicarbonate, acetic acid, and water. The substances released during this treatment will be referred to as the "pH 0.8" conjugates.

Column Chromatography

Neutral Woelm† activity 1 alumina was converted to activity 3 by shaking 15 g with 0.9 ml of water for 1 hour. A slurry of the adsorbent in benzene was poured into a 1-cm internal diameter column and the column of adsorbent was adjusted to ca. 20 cm by gently tapping the wall of the column. Gradient elution was carried out as previously described (6). The reservoirs contained

*Obtained from Worthington Biochemical Co., Freehold, New Jersey.
†Distributed by Alupharm Chemicals, P.O. Box 755, New Orleans, La.

4% ethanol in benzene, and benzene respectively. The column was operated at a flow rate of ca. 1.3 ml/minute. Eighty 10-ml fractions were collected, and then 4% ethanol in benzene was used to elute residual steroids.

Suitable aliquots of the residues representing the various steroid conjugate fractions were leached at room temperature with several small volumes of acetone and the acetone removed with a stream of nitrogen. The residue was dissolved in a small amount of benzene and applied to the adsorbent. A glass-wool plug was placed on top of the adsorbent in order to minimize plugging by insoluble material.

The solvent was removed from the individual or pooled fractions with the aid of a stream of nitrogen and a water bath maintained at 59° C. The 17-oxygenated steroids were determined by the Bongiovanni modification of the Zimmermann reaction. In this method N-benzyl trimethylammonium methoxide is substituted for KOH. The reaction was carried out at 4–8° C for 5 hours. The λ_{max} was 515 mμ and O.D. values were also read at 435 and 595 mμ, and Allan's correction was applied. When the Zimmermann reagent was used as a spray for paper chromatograms, one part of 40% N-benzyl trimethylammonium methoxide was mixed with 1 volume of ethanol and 2 volumes of a 2% ethanolic solution of metadinitrobenzene (7). The color was developed first at room temperature and then with heat. The method was sufficiently sensitive to detect 0.6 μg of dehydroepiandrosterone which had been run as a spot in the heptane – 80% methanol solvent pair.

Paper Chromatography

Paper was washed in a Soxhlet apparatus for long periods with acetic acid, ammonium hydroxide, and methanol as previously described (1). The paper was then washed with methanol for 48 hours by descending chromatography.

Hexane - propylene glycol (50% in MeOH) (HP-50), heptane:benzene (1:1) – 70% methanol (HM-70), toluene – 70% methanol (TM-70), and heptane – 80% methanol (HM-80) were used as solvent systems.

Solvents and Glassware

Dichloromethane was freshly distilled and stabilized with methanol. Methanol was added periodically during the removal of dichloromethane from steroid extracts. All other solvents were distilled. Glassware was washed with acid – chromic oxide cleaning solution followed by water, versene in aqueous methanol, and distilled water.

Sulphuric Acid Chromogens

Sulphuric acid (36 *N*) was added to the steroid to give a concentration of ca. 10 μg/ml. The spectra of the chromogens were determined after 2 hours, from 210–600 mμ, with the aid of a Beckman DK-1 spectrophotometer.

Infrared

Infrared spectra were recorded with a Beckman IR-4 double-beam spectrophotometer equipped with a beam-condensing system. Potassium bromide

(5–10 mg infrared grade) was spread over an area of ca. 0.5 inch^2 in a mortar warmed on a microheater to a temperature sufficient to volatilize dichloromethane. The steroid (ca. 20–80 μg) was taken up in 0.05–0.1 ml of dichloromethane and dispersed on the potassium bromide which was then gently mixed with a pestle. The pellet was pressed in the usual manner.

Experimental and Results

The glucuronide ("G") fraction from 650 ml of male plasma was chromatographed by gradient elution. Several appropriate standards (Table I) were treated in the same manner and quantitatively determined with the Zimmermann reagent. While it must be remembered that all Zimmermann-positive

TABLE I

Gradient elution of steroids from alumina*

Steroid	Tube No.
β-Etiocholanolone (50 μg)	10–13
Dehydroepiandrosterone (60 μg)	17–24
17β-OH-Androstan-3,11-dione (50 μg)	26–31
Isoandrosterone (100 μg)	26–32
Androsterone (70 μg)	33–38
Testosterone (200 μg)	34–39
Etiocholanolone (200 μg)	39–48
3β-OH-Androstan-11,17-dione (100 μg)	42–48

*See Method section of text for details.

steroids are not equally chromogenic, the results can be expressed as dehydroepiandrosterone equivalents, on which basis tubes 2–4 contained approximately 60 μg; 5–10, 35 μg; 14–15, 15 μg; 19, 10 μg; and 25–39, 100 μg. Tubes 2–4 of the 4% ethanol – benzene eluate contained a small amount of Zimmermann-positive material. In this paper we shall be primarily concerned with the material in tubes 25–39 which produced a Zimmermann chromogen reaching peak intensity (visual) in ca. 3 hours.

The "G" fraction from 860 ml of male plasma was chromatographed as above and the contents of tubes 25–39 were pooled and a sulphuric acid chromogen was run on one-half of the residue remaining after removal of the solvent. The chromogen had an absorption maximum at 308 mμ and a shoulder at 410 mμ. There were 60 μg of testosterone equivalents in these tubes calculated on the intensity of the absorption at 308 mμ. The remainder of the residue was applied to paper as a spot and chromatographed in the HM-80 solvent system for 3 hours. A spot, which absorbed ultraviolet light, was observed at 4.1–5.6 cm corresponding to testosterone and 17α-hydroxyprogesterone standards. The chromatogram was sprayed with the Zimmermann reagent but no chromogens developed until after the paper was heated. These experiments exhausted the supply of this fraction from male plasma.

The "G" fraction from 410 ml of plasma taken from Chilko Lake females was chromatographed using gradient elution. The residue from the contents of

tubes 25–39 was chromatographed in the HM-80 solvent system for 2.5 hours and an ultraviolet-absorbing material was present which had a mobility similar to the material obtained from male plasma. The substance was eluted from the paper and it had a λ_{max} in methanol at 238 mμ. The substance was rechromatographed in the same solvent system for 15 hours. The ultraviolet-absorbing material from plasma and testosterone both had R_t = 0.93 cm/hour, while the R_t values of 17α-hydroxyprogesterone and 11β-hydroxy-Δ^4-androsten-3,17-dione were 0.75 and 0.27 cm/hour, respectively. The plasma steroid was recovered from the paper, acetylated, and chromatographed for 2 hours in the HM-80 solvent system. The plasma substance and testosterone acetate both had R_f = 0.79. The reaction was essentially quantitative for both substances since no testosterone was detected after acetylation.

More plasma was processed and the steroid–acetate from a total of 525 ml was taken for the determination of the infrared spectrum (Fig. 1). The steroid–acetate recovered from the KBr pellet was equivalent to 2.5 μg/100 ml of plasma.

The steroid–acetate from plasma and testosterone acetate were hydrolyzed with methanolic NaOH and the steroids treated with chromic oxide. The steroids were recovered from the oxidation mixtures and chromatographed along with Δ^4-androsten-3,17-dione in the HM-80 solvent for 2 hours. All steroids had R_f = 0.30 and were detected both by their absorption of ultraviolet light and with the Zimmermann reagent.

Plasma (180 ml) obtained from Cultus Lake females was extracted with ethyl acetate and the "G" fraction obtained. The residue was chromatographed on paper for 24 hours using the HM-80 solvent system, and the region corresponding to testosterone eluted and rechromatographed in the same manner. Testosterone was determined quantitatively by ultraviolet absorption employing Allan's correction. The material from plasma exhibited maximum absorption at 238 mμ and gave O.D. readings at 220, 240, and 260 of 0.460, 0.870, and 0.291, respectively. There was 7.6 μg of testosterone released by β-glucuronidase from 100 ml of plasma based on the recovery of authentic testosterone through the procedure.

The "free" steroid fraction was prepared from 660 ml of plasma obtained from spawned Chilko Lake females. The residue was chromatographed in the HP-30 solvent system and the area of paper which would contain 17α-hydroxyprogesterone and testosterone was extracted with methanol. The methanol was removed, water added to the propylene glycol, and the steroids extracted with dichloromethane. The solvent was removed and the residue contained 90 μg of 17α-hydroxyprogesterone equivalents as determined by ultraviolet absorption employing Allan's correction. The residue was acetylated and chromatographed in the HBM-70 solvent system. There were two substances from the plasma strip which absorbed ultraviolet light. One substance (R_f = 0.6) had the mobility of 17α-hydroxyprogesterone and the other (R_f = 0.88) corresponded to testosterone acetate. Visual observation suggested that two

substances were present in comparable quantity. The infrared spectrum of the substance identified as testosterone is shown in Fig. 1.

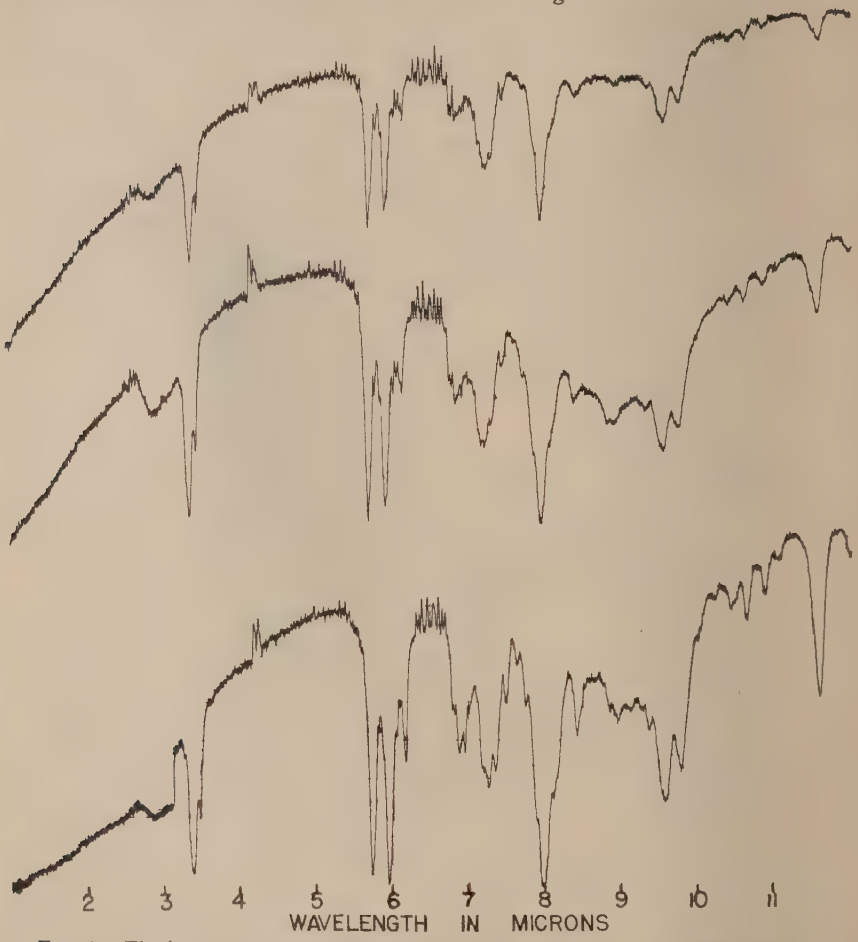

FIG. 1. The bottom spectrum is the transmittance of testosterone acetate. The top spectrum is of the acetate of the steroid obtained from the free steroid fraction, and the middle spectrum is of the acetate obtained from the conjugated fraction of plasma taken from spawned female sockeye salmon.

Several attempts were made to obtain evidence for the occurrence of dehydroepiandrosterone (DHA) or androsterone in salmon plasma. The "G" and "SO_4" fractions from 218 ml of plasma from spawned Chilko Lake females were chromatographed as streaks in the HP-50 solvent system. The areas of the paper corresponding to DHA and androsterone were eluted and the residues chromatographed as spots. No Zimmermann-positive material was detected. The "G" fraction from 136 ml of plasma taken from spawned Chilko Lake

males was treated in a similar manner and again no material with the mobility of either DHA or androsterone was detected. The "SO_4" fractions from 544 and 136 ml of the same plasma also contained no detectable DHA or androsterone. The "pH 0.8" fraction from 136 ml and the "SO_4" fraction from 47 ml of the same plasma also showed no evidence of DHA or androsterone. The "G" fraction of the plasma from 412 ml of spawned Chilko Lake females was chromatographed by gradient elution. The contents of the tubes, which should have contained DHA and androsterone had they been present, were chromatographed in the HM-80 solvent system and no ultraviolet-absorbing or Zimmermann-positive substances were detected. The "SO_4" conjugate from the plasma (870 ml) of spawned Chilko Lake females was chromatographed on a 15-cm strip of paper in the HP-50 solvent system. The Zimmermann reaction was carried out on 10% of the eluate from paper strips taken from the areas which would be occupied by DHA or androsterone and no detectable chromogen was produced. The remainder of the material with the mobility of androsterone and 25% and 10% respectively of the material corresponding in chromatographic mobility with DHA and testosterone were rechromatographed as spots in the same solvent system and no Zimmermann-positive material was detected.

The "G" and "SO_4" fractions from 224 ml and 560 ml of plasma, respectively, taken from females captured at Siwash Bridge were chromatographed by gradient elution. The Zimmermann reaction was carried out on the contents of tubes 1–80 and only tube 2 contained a significant amount of chromogenic material. Another aliquot equivalent to 47 ml of plasma was chromatographed in HP-50 alongside 17α-hydroxyprogesterone dehydroepiandrosterone, androsterone, and etiocholanolone. There was a faint Zimmermann-positive spot with the mobility of 17α-hydroxyprogesterone (or testosterone) and nothing corresponding to the other reference substances. The "pH 0.8" conjugates prepared from 170 ml of plasma taken from spawned Chilko Lake males were subjected to column chromatography. Only a trace of Zimmermann-positive material was detected.

Discussion

The failure, in the present study, to detect either DHA or androsterone in any of several plasma samples is of some interest since both these steroids are present in relatively high concentration in human peripheral plasma. Dehydroepiandrosterone has been isolated and positively identified in human plasma where it is reported to occur at a concentration of 57.5 ± 10.5 μg/100 ml (8). Androsterone has been reported to occur at a concentration of 18 μg and 22.5 μg/100 ml, respectively, in the plasma of normal females and males (9).

The first demonstration of the conjugation of a steroid at a position other than the C(3) hydroxyl group was the isolation of testosterone-17β-glucuronide from the urine of a patient receiving large doses of testosterone propionate (10). Testosterone is converted in low yield by rat liver slices in vitro to a substance positively identified as testosterone-17β-glucuronide (11). Unconjugated testo-

sterone has recently been isolated from human systemic blood following the administration of human chorionic gonadotrophin (12). To our knowledge conjugated testosterone has not been previously isolated from blood.

There was 7.6 μg/100 ml of testosterone in the "G" fraction of plasma taken from spawned Cultus Lake females. In another experiment 2.5 μg/100 ml of testosterone was recovered from 525 ml of plasma taken from spawned Chilko Lake females. This substance had been taken through two paper chromatograms, one column chromatogram, acetylation, and recovery from a KBr pellet. The recovery from each paper chromatogram can be estimated from the recovery of pure steroids to be ca. 85%. Since no testosterone was detected on the paper chromatogram following acetylation the recovery was ca. 95%. The recovery from the KBr pellet and column chromatogram can only be estimated approximately, ca. 85% each. There were therefore ca. 5.1 μg of testosterone released from each 100 ml of plasma by β-glucuronidase. This estimated level of testosterone in the spawned Chilko Lake females was therefore comparable to the level accurately determined in the "G" fraction of plasma obtained from Cultus Lake spawned females.

The plasma sample obtained from females captured at Siwash Bridge, approximately 3 weeks prior to spawning, contained no detectable Zimmermann-positive substance in tubes 4–80 following column chromatography. It must be remembered that the Zimmermann reaction as used in this study is quite ineffective on 17-hydroxysteroids and the intensity of the color produced with 3-ketosteroids reaches a maximum in 3 hours and has decreased in intensity by the time the tubes are read at the end of 5 hours, which is the optimum time for 17-ketosteroids. Since significant amounts of steroids with a Δ^4-3-ketone structure were not expected to occur as conjugates at the time these chromatograms were run testosterone, unless present at a concentration of ca. 2 μg/100 ml, would probably not have been detected. However, testosterone should have been detected by paper chromatography if it had been present in significant quantities. It has been shown in a separate investigation that this plasma sample contained only 3.8 μg/100 ml of "free" 17α-hydroxyprogesterone plus testosterone, while the plasma of spawned Cultus Lake females, which contained 7.6 μg of testosterone in the "G" fraction, contained 7.7 μg/100 ml of testosterone and 9.8 μg/100 ml of 17α-hydroxyprogesterone in the "free" steroid fraction. The steroids were determined following paper chromatography in the heptane – 80% methanol solvent system for 16 hours (13). It can be concluded that testosterone was present at a considerably higher concentration in both the "G" and "free" fractions of spawned females than in the corresponding fractions prepared from the plasma of females captured prior to arrival in the spawning area. Testosterone in the combined "free" and "G" fractions of plasma from spawned Chilko Lake females was 15.3 μg/100 ml (7.7 μg plus 7.6 μg). By contrast the level of "free" testosterone in normal human peripheral plasma of both sexes is extremely small and values ranging from 0.1 μg to 0.4 μg/100 ml have recently been reported (14). It can also be

concluded that testosterone is present at a considerably higher concentration in both the "G" and "free" fractions of spawned females than in the corresponding fractions prepared from the plasma of females captured prior to arrival on the spawning grounds.

17α-Hydroxyprogesterone was previously isolated from the "free" steroid fraction of plasma taken from females en route to Adams River (1). Testosterone and 17α-hydroxyprogesterone were both isolated from the "free" steroid fraction of plasma taken from spawned Chilko Lake females in the present study. Testosterone and 17α-hydroxyprogesterone are difficult to separate by paper chromatography although the HM-80 solvent system will effect an adequate separation if run long enough (i.e., 16 hours). The fact that testosterone acetylates under very mild conditions, while 17α-hydroxyprogesterone does not, forms the basis for the separation used in this study. The distribution of the two "free" steroids in plasma samples taken from fish at various stages of sexual maturity has been studied and the data will be included in a separate submission (13).

Acknowledgments

One of the authors (D.G.) wishes to gratefully acknowledge generous financial assistance from the International Pacific Salmon Fisheries Commission while carrying out this research in partial fulfillment of the requirements for a Master's degree in Zoology from the University of British Columbia. The authors wish to thank Dr. P. A. Larkin, Head of the Institute of Fisheries, University of British Columbia, for permitting Mr. Grajcer to carry out this research in these laboratories.

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OLFACTORY PERCEPTION IN MIGRATING SALMON

II. STUDIES ON A LABORATORY BIO-ASSAY FOR HOMESTREAM WATER AND MAMMALIAN REPELLENT[1]

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Abstract

A laboratory bio-assay, based on the response of adult migrating sockeye salmon (*Oncorhynchus nerka*) to homestream water, is described. A characteristic response to the repellent in mammalian skin is discussed. The homestream substance(s) was tentatively identified as volatile, dialyzable, neutral, and heat-labile.

The transportation and holding of sockeye in captivity was investigated.

Introduction

Many studies have clearly demonstrated the keen olfactory perception of fish (1, 2, 3, 4, 5, 6). Salmon respond to a repellent in mammalian skin at extremely high dilution (7, 8) and the young eel can be trained to detect 1 part of β-phenyl ethylalcohol in 3×10^{18} parts of water (9).

The ability of salmon species to return to spawn in the stream of their birth is adequately documented and reviewed (10, 11). For example, nearly one-half million sockeye (*Oncorhynchus nerka*) fingerlings were marked and released in a Fraser River stream and 11,000 adults returned to the parent stream but none to nearby tributaries (12).

In another instance, a marked steelhead was released in the Alsea River, Oregon, in April, 1958. The fish was captured and tagged near Alaska on September 5, 1958. It was recovered back in the Alsea River on February 5, 1960. This fish made a minimum round trip of 2400 miles to return to the stream of its birth (13).

An interesting question was examined by Higgins (14). Given a choice, will salmon return to the homestream of their parents or to the water in which they spent their first months of life? Higgins transplanted sockeye eggs from Baker River, a tributary of the Skagit River in Washington, to Grandy Creek, a stream located above Baker River but emptying into Skagit River. The hatched fry from these transplanted eggs were marked. In due course it was noted that marked adults returned only to Grandy Creek. In 1949, scientists of the International Pacific Salmon Fisheries Commission investigated this question further by transporting fertilized sockeye eggs from the Horsefly River and hatching them in the Quesnel Field Station on Horsefly Lake (15). In November, 1950, the fingerlings were transported by air downstream to Quesnel Lake at the mouth of the Horsefly River. In 1953 only one marked fish was

[1]Manuscript received March 9, 1961.
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recovered in the homestream of the parent stock, while 203 marked adults were recovered in the area of the hatchery. Thus, the fish returned to their homestream water by a route which they had never before travelled.

Craige's pioneering study (16) and Wisby and Hasler's (17) experiments lend strong support to a homestream odor theory. In the latter work, sexually ripe coho salmon were captured in their homestream on a branch of the Isaquah River, Washington. They were returned downstream below a fork and the nasal sacs of half of the fish were plugged with cotton while the remainder were untreated. The untreated fish returned again to the stream of their first choice, while the treated fish returned in a random fashion to the two tributaries (17).

The goals of the present studies were to develop laboratory bio-assays to facilitate the isolation and identification of the homestream odor(s) and to permit further study of the salmon repellent in mammalian skin (7, 8).

Methods and Materials

Selection of Fish

In selecting the species of salmon to be used, careful consideration was given to the following factors: the migratory routes of the fish and their accessibility for fish trapping and water sampling; the migratory behavior pattern of the fish and its suitability for study in the limited space of aquaria; the relative inherent resistance of the different species of salmon to disease, i.e. the adaptability and survival of the fish in captivity. Information on the relative resistance of the various species of adult salmon to disease is scant. Experience of fish culturists has indicated that the coho can be held in captivity for periods of up to several months. While the coho and spring salmon show strong territorial behavior, the sockeye, pink, and chum display schooling behavior. The latter behavior pattern would be the more desirable in the confined space of aquaria. While both the pink and the chum vary greatly in their weights and lengths at maturity, the adult sockeye of a given race is uniform in both weight and size. It thus appeared that the salmon species best suited for the study was the sockeye, providing suitable conditions for holding the fish in captivity could be worked out.

The sockeye is abundant along the whole west coast from Alaska to California. The life cycle varies somewhat between localities. Once the yolk sac is absorbed, the fingerling usually spends 1 year in the lake and then migrates to sea during the spring. In certain areas, however, the fingerling may spend 2 or 3 years in fresh water before beginning the seaward migration. Most sockeye mature after having spent 3 to 4 years at sea. While information concerning the movements of sockeye at sea is meager, the migratory routes of these salmon in the major river systems have been studied extensively with the use of tags, traps, and counting fences. In general, the weight of the mature sockeye is between 5 and 7 pounds. Adult sockeye cease to feed when they enter fresh water for their spawning migration. While both sexes are similar in external appearance at the start of the spawning migration, the external appearance of

the male changes dramatically a few weeks prior to spawning. The skin of the male changes in color from a dull grey-green to brilliant red. Also, the male develops a distinct hump and a pronounced hooked snout. The major noticeable external change in the female from the start of the spawning migration to the time of spawning is a loss in the brilliance and coloring of the skin and a lesser elongation of the snout. Sockeye, whether as fry migrating to sea or as adults migrating to the spawning beds, school constantly.

Handling of Fish

In order to minimize bruising and loss of scales while transferring the fish from the trap to the transport tank and to aquaria, only wet wool gloves and knotless nylon nets were used. If the transport distance exceeded 40 feet the fish were carried in a 1 ft × 1 ft × 6 ft aluminum tank filled with water. To avoid injury to the fish during these short transfers, an anaesthetic, methane tricaine sulphonate (MS 222), was added to the water at a level of 1:50,000. This concentration of anaesthetic induced a mild sedation which minimized struggling and subsequent injury to the fish.

Transport

The transport tank used to transfer the fish from the field sites to the aquarium was a circular 400-gallon aluminum tank 4 feet in diameter and 3 feet in height with a conical top. A bilge pump placed at the bottom of the tank gave continuous water circulation at the rate of 3 gallons/minute. The water outlet from the pump, situated at the top of the tank, was fitted with a sieve to disperse the water in a fine spray. This dispersion of water not only aided circulation but also facilitated aeration. As an added precaution to ensure maximum available oxygen and minimum carbon dioxide build-up in the water, the top of the tank was perforated with numerous small holes to allow free air passage in and out of the tank. In addition, oxygen was passed into the water from a pressure cylinder. The water was isotonic with respect to sodium chloride. To placate the fish, MS 222 was added to the water at a level of 1:100,000. This concentration of anaesthetic induced a very mild form of sedation from which the fish recovered quickly when transferred to fresh water. The water temperature during transport was held at 7° to 9° C. During the summer it was found necessary to add ice to the water in order to maintain this temperature. The ice was prepared from chlorine-free water. To minimize the risk of infection, chloromycetin was added at a level of 25 mg per gallon of water. Under these conditions, a total of 250 sockeye were transported distances of up to 250 miles without mortality. The largest single load was 50 fish.

Holding and Experimental Tanks

Two 6 ft × 3 ft circular aquaria constructed of plywood, coated on the inside with fiberglas, and having a 1-ft center core, were used to hold fish which were not under immediate study. At Courtenay, B.C., the fish were held in outdoor concrete ponds (36 ft × 6 ft × 4 ft) with adequate water flow. The experimental aquarium, 14 ft × 3 ft × 3 ft, was constructed of cement and the front plate of

2-in. photographic glass. Each reserve tank had a capacity of 300 gallons and the experimental tank 757 gallons. In order to conserve test materials, the experimental tank contained only 400 gallons of water during use. All water was dechlorinated and carried via iron or plastic pipe. Water was supplied to the reserve tanks at a rate varying between 8 and 10 gallons/minute and to the experimental tank at 3 to 4 gallons/minute. Each tank was aerated with spargers.

Source of Fish

When captured, all fish were actively engaged in spawning migration. The sockeye employed in the initial phase of this study were obtained during July, 1960, at the outlet to Great Central Lake. This water system located in the Alberni area of Vancouver Island, B.C., supports a well-established sockeye population. These fish showed none of the external changes associated with sexual maturity. They normally spawn in late October and November. The second group of fish were obtained during early November at Sweltzer Creek, the outlet to Cultus Lake. This watershed located in the southern interior of British Columbia also supports a sizable sockeye population. These fish when captured were advanced in their sexual development and would normally spawn in 4 to 6 weeks.

Treatment of Fish

Disease, if allowed to progress unchecked, will bring about significant changes in the behavior pattern of fish (18). The migrating sockeye is particularly vulnerable to fungus (*Saprolegnia parasitica*) infection, especially if superficial wounds exist or if water temperatures are excessive, i.e. greater than 12° C (18). This fungus growth when left untreated progresses rapidly and in 2 to 3 weeks can not only kill the host but also infect other fish. The success of these studies depended upon finding a suitable treatment to prevent disease, particularly fungus. In preliminary experiments carried out at Courtenay, B.C., and the Vancouver Public Aquarium, various treatments were evaluated. Malachite green (18) was used at a concentration of 1:100,000 for 1 hour at 9° C, and the fish survived. However, the fungus continued to spread in spite of a second treatment 4 days later. Fish did not survive a 1.5-hour treatment at a concentration of 1:250,000 and a water temperature of 13 to 14° C on several occasions.

Salt baths, when given bi-weekly for a period of 3 hours at a level of 3% NaCl, were found to be very effective at water temperatures of 8–10° C in not only controlling the spread of fungus but also for the most part in preventing the appearance of the disease. This treatment was also effective at Courtenay where the water temperatures often fluctuated 5° C in a 12-hour period and reached an average daily high of 17.5–19.5° C for a period of several weeks. At Courtenay the treatment was given every 2 days. There were no deaths in a group of 50 sockeye between July 10 and August 17. If fish were wounded externally they often reacted violently to the salt bath and there was danger of further injury. To alleviate this risk the fish were first anaesthetized to a state

of mild sedation by the addition of 2-phenoxyethanol (reported to be antifungal (18)) to the aquarium water at a level of 20 ml/40 gallons and then treated with salt.

Testing Water Samples

The regular water supply entered the experimental aquarium at one end through rubber tubing, the outlet of which was placed one foot below the water surface. Air was supplied to the aquarium through a 3 ft × $\frac{3}{4}$ in. rubber hose fastened across the bottom of the tank. A series of $\frac{1}{8}$-in. holes in the face of the hose gave a blanket of air which created a current across the top of the tank down the opposite end and across the bottom in a clockwise direction. Water samples to be tested for their effect on the fish were injected into the aquarium through a separate rubber hose connected at one end to a bilge pump seated in a large plastic bucket and then run parallel to the water line into the tank. The delivery rate of the test water varied between 2.8 and 3.0 gallons/minute.

Tests with potassium permanganate showed that the time interval for distribution of the compound throughout the whole of the tank was 2 minutes and 10 seconds from the time of injection. It required 6 hours and 20 minutes to clear all visible traces of permanganate from the tank. The entire segment of the room in which the experiments were performed was partitioned off with black plastic so that any movements of workers were invisible to the fish. The tank received illumination only from above. A peephole large enough to accommodate a camera lens was cut in the curtain at a point directly opposite the center of the glass side of the tank. The distance from the center of the glass to the peephole was 12 feet. A sheet of plywood which extended to the roof was mounted across the inlet end of the tank. The plywood supported the water lines and also permitted the operation of the various taps without disturbance of the fish. To encourage the fish to school at the end of the tank opposite to the test sample inlet, a shaded area was produced by placing black polyethylene over the extreme one-third of the tank opposite to the water inlet. A constant illumination of 150 ft-c in the center of the lighted area and 55 ft-c in the shaded area at the respective exposed water surfaces was provided by a single 500-watt lamp. The activity of the fish was recorded with a 16-mm Ciné camera.*

Exploratory studies showed that a minimum of 9 gallons of homestream water was required to produce a consistent response by the fish. A similar volume of Capilano River water evoked no response. The activity of the fish was watched for at least 15 minutes preceding, and 20 minutes following, the injection of the test water. Filming was begun 1 minute before, and continued for 7 minutes after, the start of the injection. The time required for injection was usually between 4 and 5 minutes. To obviate the possibility of contaminating the test waters with human repellents, plastic or rubber gloves were used whenever the test waters were handled (7, 8). The water samples were stored at 5° C in 4-gallon cans. The activity noted prior to the injection served as the

* Arriflex 16. Manufactured by Arnold and Richter, 89 Tuerkenstr., Munich 13.

control for the response obtained, if any, after the injection was started. If a response was obtained it usually occurred 1 or 2 minutes after the injection was completed. To ensure complete removal of test waters from the aquarium an interval of at least 24 hours was allowed between successive tests. On days when salt baths were given, the test was carried out preceding the salt treatment. Predominantly female fish were used and were changed frequently to avoid conditioned responses.

The water sample was said to elicit a positive response when (1) the school was dispersed, (2) the swimming speed of the fish increased, and (3) the fish did not reform the school for at least 3 minutes. Occasionally the fish broke the surface of the water. Where a positive response was obtained to a test water sample, the fish remained active for a period of from 10 to 40 minutes. This active period was sometimes punctuated by brief moments of schooling but not necessarily in the shaded area which the fish occupied during the control period.

The repellent used in this study consisted of about 2 liters of water in which two persons had washed their hands. The response in this test is quite different from that to the homestream water. In the response to the repellent, the fish sometimes show a little activity after the introduction of the repellent and then they move to the left-hand corner of the tank where they stay for several minutes. They school very closely and actually swim backwards into the corner. This behavior was observed only during the response to the repellent. Occasionally a fish moved a short distance from the school but returned very quickly.

Results

All the responses of the Great Central Lake and Cultus Lake sockeye to the different waters are recorded in Tables I and II and the sources of these samples are shown in Figs. 1 and 2.

TABLE I

Reaction of Great Central Lake fish to various waters*

	Great Central Lake	Lindsay Creek	Drinkwater Creek	Forest Camp Creek	Sweltzer Creek	Weaver Creek	Hand Rinse
Untreated water	++++ ++++	++	++	−−	−−−	−	+++
Lyophylized† volatile	+						
Lyophylized residue	−				−		
Ion-exchanged‡ water	+				−		
Autoclaved§	−						
Non-dialyzable‖ fraction	−						

*Each + or − represents one test. All test results are shown.
†Condensing flask held in dry ice – acetone bath; sample concentrated in water bath at 25° C to 30° C.
‡Resin column 30 cm in length and 3.5 cm in inside diameter. Ion exchange carried out at 5° C using Amberlite IR 120(H) and IRA 400 (OH).
§Steam heated at 100° C for 3 hours.
‖Dialyzed against running tap water (8–10° C) for 72 hours.

TABLE II

Reaction of Cultus Lake fish to various waters*

	Sweltzer Creek	Spring Creek	Frost Creek	C.L. Hatchery Creek	Weaver Creek	Steelhead Creek	Great Central Lake
Untreated water	+++	++	−−	++	−	−	−
Lyophylized† volatile	++						
Lyophylized residue	−						
Autoclaved‡	−						
Non-dialyzable§ fraction	−						

*Each + or − represents one test. All test results are shown.
†Condensing flask held in dry ice – acetone bath; sample concentrated in water bath at 25° C to 30° C.
‡Steam heated at 100° C for 3 hours.
§Dialyzed against running tap water (10° C) for 72 hours.

Great Central Lake fish responded positively to Great Central Lake water in eight separate tests (Table I). The water samples were taken at the outlet to the lake. The fish also responded positively to the water from Lindsay Creek and Drinkwater Creek, both of which feed a beach spawning area. There is also a small run of sockeye into Drinkwater Creek. The fish did not respond to the water from Forest Camp Creek, which to our knowledge does not support a sockeye population. Sweltzer and Weaver Creeks both have sockeye populations but are on the mainland and as would be anticipated the Great Central Lake fish gave no response to these waters. When Great Central Lake water was lyophilized at low temperature the fish responded to the volatile fraction but did not respond to the residue. When the same water was distilled under reduced pressure (water pump) with heating to about 70° C, the fish did not respond to either the distillate or to the residue. After Great Central Lake water was autoclaved at a 100° C for 3 hours the fish no longer responded to it.

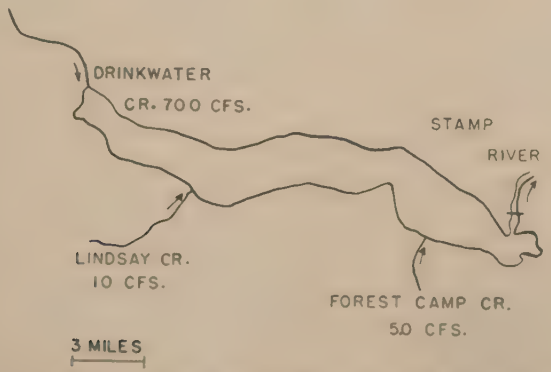

FIG. 1. Location of creeks feeding Great Central Lake, B.C., from which water was tested. Flow rates at time of adult sockeye migration.

Great Central Lake water was passed through a mixed bed of strong base and strong acid ion-exchange resins and the effluent produced a positive response. Great Central Lake water was dialyzed for 72 hours against running tap water at 8–10° C. The fish did not respond to the non-dialyzable material. Sweltzer Creek water was also passed through a mixed bed ion-exchange resin in order to determine if anything was eluted from the resin which may have been responsible for the positive response of Great Central Lake water treated in a similar fashion. The result was negative (Table I).

Cultus Lake fish responded positively to water from Sweltzer Creek through which they must pass in order to reach the Lake (Table II). They also responded positively to water from Spring Creek which feeds the main spawning area at the south end of the Lake (Fig. 2). Fish are also reported to spawn in

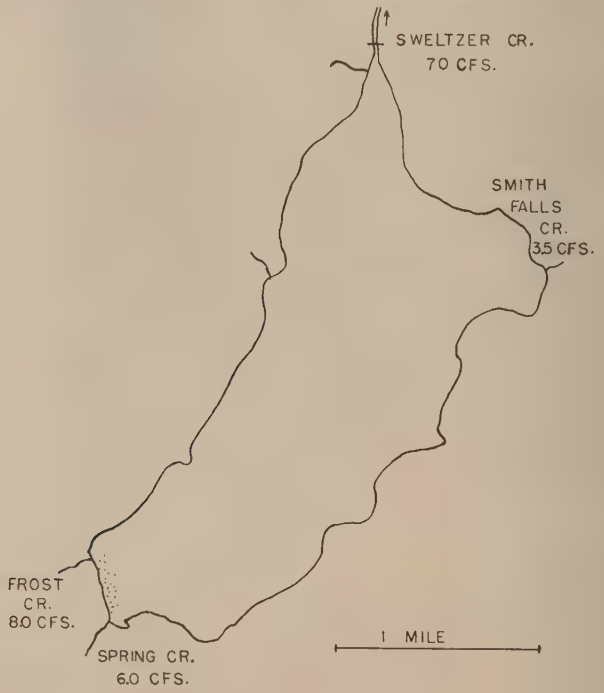

FIG. 2. Major creeks feeding Cultus Lake, B.C. Flow rates at time of adult sockeye migration.

the vicinity of Hatchery Creek and this water also elicited a positive response. The results on Frost Creek are of some interest, as this creek is very close to Spring Creek. However, there is no sockeye run into Frost Creek, whereas there is a small sockeye run into Spring Creek. The fish did not respond to the water from Frost Creek. Weaver and Steelhead Creeks are part of the Harrison River system, which is in close proximity to, but separate from, Cultus Lake.

Weaver Creek has a fairly large sockeye run, while Steelhead is barren. The Cultus Lake fish did not react to the water from either creek, nor did they react to the water from Great Central Lake. The responses of Cultus Lake fish to various fractions of Cultus Lake water confirmed the results obtained with corresponding fractions of Great Central Lake water tested on Great Central Lake fish, except that ion-exchanged water was not tested on Cultus Lake fish. Great Central Lake fish were repelled on three separate occasions by a rinse of human hands (Table I).

Many proposals have been made as to the type of clues by which salmon are guided to their spawning grounds. The effect of temperature (20) and CO_2 tension (21) in the selection of homestreams have been widely supported in the past, but with recent findings these proposals, for the most part, have been discarded (10, 15) in favor of chemical stimuli as the guiding factor in the selection of migratory routes at least during the fresh-water phase of the spawning migration.

It is acknowledged that the data presented here are based on a limited number of observations, particularly the fractionation studies. They are being presented at this time because the seasonal nature of the work will permit no further investigation until midsummer of 1961. With this in mind it is possible to draw certain tentative conclusions. The unknown active component (S) is water-soluble, neutral, dialyzable, and heat-labile in Great Central Lake water, while in Cultus Lake water it has been shown to be water-soluble, dialyzable, and heat-labile. Considering the high dilution of the samples, it is extremely unlikely that either CO_2 or water temperature would contribute to the responses obtained.

Acknowledgments

Dr. R. A. MacLeod, formerly at these laboratories and now at the Department of Bacteriology, Macdonald College, Que., collaborated in the formulation phase of this project. Scientists of the International Pacific Salmon Fisheries Commission, Federal Department of Fisheries, B.C. Fish and Game Department, and the Nanaimo Biological Station of the Fisheries Research Board of Canada rendered valuable assistance. Dr. M. A. Newman, Curator of the Vancouver Public Aquarium, provided laboratory space for holding and testing fish.

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THE EFFECT OF DIET, AGE, AND SEX ON CHOLESTEROL METABOLISM IN WHITE LEGHORN CHICKENS[1]

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Abstract

The cholesterol metabolism of chickens differed in birds of different age and sex. The normal serum cholesterol levels were higher in females than in males and higher in 1-week-old chicks than in mature birds of the same sex. Laying hens were less susceptible to hypercholesterolemia induced by dietary cholesterol than were mature cockerels or young chicks of either sex. Corn oil tended to increase the degree of hypercholesterolemia in all cholesterol-fed birds except young cockerels. A vitamin-A-rich oil, dogfish liver oil, decreased the rise in serum cholesterol level in cholesterol-fed chicks of both sexes, but in mature birds it produced the opposite effect. The addition of 1% cholesterol to the control diet of hens increased the yolk cholesterol concentration of eggs laid by the birds and this increase in concentration was greatly enhanced if 10% corn oil was also present in the diet.

Introduction

Previous work in this laboratory (1) showed that the inclusion of certain fish oils in the diet prevented the onset of hypercholesterolemia induced in young cockerels by cholesterol feeding. The major active factor in the oils was later identified as vitamin A (2). On the other hand, Weitzel *et al.* (3) administered large oral doses of vitamin A to old atherosclerotic hens, and although a marked anti-atherosclerotic effect was observed, the change in the serum cholesterol level was slight. The dissimilar effects of the vitamin could be due to differences in the two investigations which are too numerous to discuss here in detail, but one possibility is that the cholesterol metabolism of chickens is influenced by their age and sex.

That age and sex do have a bearing on serum cholesterol levels of poultry is indicated by the results obtained by several workers. Lorenz *et al.* (4) observed that laying hens had higher blood cholesterol levels than immature hens or mature roosters, and Rodbard *et al.* (5) found that the degree of hypercholesterolemia in cholesterol-fed cockerels depended on the age of the birds. Stamler and co-workers (6) showed that laying hens had to be fed twice as much cholesterol as that given to roosters to obtain the same elevation of serum cholesterol levels.

In view of the above observations the present investigation was initiated to study dietary alteration of serum cholesterol levels in young and old chickens of both sexes. At the same time the effect of the different diets on cholesterol content of eggs was studied.

[1]Manuscript received April 27, 1961.
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The effect of diet on the composition of eggs has been studied by numerous workers. McCollum *et al.* (7), Cruickshank (8), and more recently Reiser (9) and Fisher (10, 11) showed that the composition in egg yolk could be altered by diet but in all cases it was the type of fatty acid in the yolk which was changed. The cholesterol content was shown in the more recent studies to be independent of the amount or type of fat in the hens' diet. The diet used in these investigations did not contain added cholesterol but findings reported by Kurnick *et al.* (12) indicated that even the presence of 1% cholesterol did not alter the cholesterol content of eggs.

Materials and Methods

Birds

White Leghorn birds were used in all experiments. The mature cockerels and laying hens were approximately 8 to 10 months old and the young birds of both sexes were obtained as day-old chicks and kept on the control diet for 7 days prior to the beginning of the tests. The mature roosters were housed in individual cages, the laying hens two to each cage, and the chicks in groups of 16. The older birds were placed in their respective cages 5 days prior to the beginning of the experiment and during this time they were fed the control diet. The studies were carried out during the fall and winter months.

Diets

In order to compare the cholesterol metabolism of birds of different ages and sexes, one should feed the birds the same diet. This is impossible in the case of chickens because of the different dietary requirements of the groups. A control diet for young birds of both sexes and for mature cockerels was formulated as shown in Table I. This diet contained 2% fat and 13% protein. The control

TABLE I

Percentage composition of control diet

Ground wheat	41.20	Distillers' dried solubles	1.87
Ground yellow corn	7.50	Dehydrated cereal grass	0.94
Ground oats	3.75	Iodized salt	0.37
Wheat middlings	3.75	Ground limestone	0.75
Wheat bran	3.75	Manganese sulphate	0.01
Soybean oil meal	5.62	Feeding oil (2250 A–300 D per gram)	0.20
Fishmeal	1.50	Nicarbazin	0.04
Meatmeal	3.75	Sucrose	25.00
Riboflavin 50 mg/100 lb feed			

diet for the laying hens had the same composition except that an additional 2% ground limestone and 0.5% bone flour was included to satisfy the laying birds' greater demands for calcium and phosphorus. Additions to the control diets listed in the tables and figures which follow were substituted isocalorically for sucrose, thereby maintaining a constant energy to protein ratio in the diet, a ratio which has been shown (13) to influence serum cholesterol levels in chicks.

Dogfish liver oil from the same batch was used throughout the investigation and its vitamin A content was 8300 I.U./g oil.

The diets were stored at 2° C and fresh feed was placed in the troughs each day to minimize oxidation. The amount supplied to the birds was adjusted so that the calorie intake was approximately the same on all diets. The test diets were continued for 14 days. The duration of the experiments, admittedly rather short, was chosen in view of the results of Rodbard *et al.* (5), which indicated that chicks' susceptibility to cholesterol-induced hypercholesterolemia varied considerably during the first 2 months of their lives. The authors therefore chose a short period (14 days) for the tests, thereby eliminating as far as possible the occurrence of changes in cholesterol metabolism during the test period. Previous experience with chicks indicated that dietary-induced changes in serum cholesterol levels were well established by 14 days.

Determination of Serum Cholesterol

The birds were starved for 18 hours prior to the collection of blood from the brachial vein in order to avoid any short-term changes in serum lipids due to recent ingestion of food by the birds. Blood samples from chicks were pooled in lots of four and the serum collected in the usual manner, but individual samples were used in the case of the mature birds. The total cholesterol concentration in the serum was determined by the method of Sperry and Webb (14).

Determination of Egg Yolk Cholesterol

Estimations were carried out on all eggs individually. The shell was cracked open and the yolk carefully separated from the white of the egg. The yolk was weighed and homogenized for 1 minute with 35–40 ml water using a Servall homogenizer and was then made up to 100 ml with water and mixed thoroughly. The resultant suspension of egg yolk settled slowly but there was ample time to procure aliquots for analysis. Moreover, a brief shaking of a sample which had been standing for several hours produced a suspension which yielded cholesterol analyses which were in close agreement with those obtained using the freshly prepared suspension. An 0.5-ml aliquot from the suspensions was run slowly into a 25-ml volumetric flask containing 10 ml acetone–alcohol (1:1 v/v). The cholesterol determinations were then carried out exactly as described by Sperry and Webb (14) for blood cholesterol.

Identification of Cholesterol and Plant Sterols

The identity of the sterols in corn oil, dogfish liver oil, serum, and egg yolk was determined as follows. Five-gram samples of oil or 10-ml aliquots of egg yolk suspension or serum were saponified with alcoholic alkali and the unsaponifiable material extracted with ether. The ether extract was washed successively with two portions of water, 0.1 *N* KOH, and water until the washings were colorless to phenolphthalein. The ether extract was evaporated to dryness *in vacuo* after most of the solvent had been removed at 60° C under a stream of nitrogen. The residual material was dissolved in ether and made up to a final volume of 10 ml. Aliquots of this solution were chromatographed on Whatman

No. 1 paper impregnated with kerosene, b.p. 220°–240° C, according to the procedure of deZotti *et al.* (15). The mobile phase was pyridine–water (85:15 v/v). The temperature was kept at 20° C instead of the 15° C recommended by de Zotti *et al.* since, unlike the latter workers, we found excellent separation at the higher temperature.

Results

Certain dietary constituents altered the concentration of total cholesterol in the serum of young cockerels (Table II). The chicks were highly susceptible to cholesterol-induced hypercholesterolemia, the serum cholesterol being increased sixfold when 1% cholesterol was included in the diet. The presence of corn oil did not alter this effect but dogfish liver oil, in agreement with previous findings, reduced the degree of hypercholesterolemia. It should be mentioned at this point that all differences in serum cholesterol level reported in this paper are statistically significant at $P = 0.01$ unless otherwise stated.

TABLE II

The effect of diet on serum cholesterol levels in 7-day-old cockerels

Diet	Food consumption (g/bird/day)	Relative calorie intake	Weight gain (g)	Serum cholesterol (mg/100 ml)
Control	16.0	100	68.1	183 ± 5*
Control + 1% cholesterol	15.4	103	63.6	1099 ± 281
Control + 1% cholesterol + 10% corn oil	14.6	106	76.0	1028 ± 152
Control + 1% cholesterol + 10% dogfish liver oil	14.4	104	56.3	435 ± 86

*Mean value for four groups of serum ± the standard deviation. Each group contains serum from four birds.

The mean weight gain of the cockerels varied considerably with the type of diet in spite of the fact that the calorie intake was approximately the same on all diets. In particular, the weight gain of birds of the fish oil diet was low. Similar low weight gains were observed in most experiments conducted in these laboratories with various fish oils possessing hypocholesterolemic activity, and these findings might suggest that the oils were perhaps toxic to growing chicks and that the associated lower cholesterol levels were a non-specific resultant thereof. However, in some experiments the rate of growth of chicks on fish-oil-supplemented diets was comparable to that of birds on the cholesterol-containing control diets and significant hypocholesterolemic activity was still in evidence (Table III). It would appear therefore that there is no definite association between the hypocholesterolemic effect and the depression in growth rate.

In young pullets the dietary regimens produced effects similar to, but not identical with, those observed in the male chicks (Table IV). The serum cholesterol level in the "control" birds was higher than in young cockerels and the hypercholesterolemic effect of 1% cholesterol was increased by the presence

TABLE III

Hypocholesterolemic activity of fish-oil-supplemented diets which support a growth rate in chicks similar to that observed with cholesterol-containing control diets

Expt. No.	Duration of experiment (days)	Addition to control diet containing 1% cholesterol	Food consumed (g/chick/day)	Weight gain (g)	Serum cholesterol (mg/100 ml)
1	10	None	16.0	48.3	686 ± 220*
		2% lingcod liver oil (42,500 I.U. vit. A/g)	13.6	46.6	476 ± 65
2	14	None	13.7	63.6	990 ± 68
		9% lingcod liver oil (18,900 I.U. vit. A/g)	14.2	65.6	179 ± 23
3	14	None	14.5	64.7	983 ± 214
		10% dogfish liver oil (8,000 I.U. vit. A/g)	13.8	63.1	556 ± 62

*Mean value for four groups of serum ± the standard deviation. Each group contains serum from four birds.

of 10% corn oil, although, due to the large variation in the individual values, this "additional" increase was not significant at $P = 0.01$. Dietary dogfish liver oil acted as a hypocholesterolemic agent and also reduced the rate of growth of the chicks, as was the case with cockerels.

TABLE IV

The effect of diet on serum cholesterol levels in 7-day-old-pullets

Diet	Food consumption (g/bird/day)	Relative calorie intake	Weight gain (g)	Serum cholesterol (mg/100 ml)
Control	17.0	100	56.2	248 ± 7*
Control + 1% cholesterol	16.6	99	58.0	847 ± 120
Control + 1% cholesterol + 10% corn oil	14.0	96	59.1	1092 ± 262
Control + 1% cholesterol + 10% dogfish liver oil	13.3	91	38.6	491 ± 144

*Mean value for four groups of serum ± the standard deviation. Each group contains serum from four birds.

The effect of diet on serum cholesterol levels in mature cockerels is shown in Table V. The cholesterol level of birds on the control diet was lower than that observed with young birds of either sex. The incorporation of 1% cholesterol

TABLE V

The effect of diet on serum cholesterol levels in mature cockerels

Diet	Food consumption (g/bird/day)	Relative calorie intake	Mean change in body weight (lb)	Serum cholesterol (mg/100 ml)
Control	122.6	100	−0.1	134 ± 19*
Control + 1% cholesterol	123.9	103	−0.1	599 ± 328
Control + 1% cholesterol + 10% corn oil	104.4	101	0.0	707 ± 312
Control + 1% cholesterol + 10% dogfish liver oil	90.8	86	−0.1	980 ± 366

*Mean value for 12 birds ± the standard deviation.

into the diet caused a 4.5-fold increase in serum cholesterol concentration. This elevated cholesterol level was increased still further by both corn oil and dogfish liver oil, but only with the latter oil was the increase, over and above that caused by the dietary cholesterol, significant. The body weight of the mature cockerels did not alter appreciably during the course of the experiment.

The cholesterol levels of laying hens on the test diets are shown in Table VI. The level in the control birds was higher than in mature cockerels but lower than in young pullets. Dietary cholesterol itself failed to increase significantly

TABLE VI

The effect of diet on serum cholesterol levels in laying hens

Diet	Food consumption (g/bird/day)	Relative calorie intake	Mean change in body weight (lb)	Serum cholesterol (mg/100 ml)
Control	116.2	100	0.0	177 ± 109*
Control ± 1% cholesterol	118.9	104	−0.1	203 ± 85
Control + 1% cholesterol + 10% corn oil	109.4	109	0.0	295 ± 143
Control + 1% cholesterol + 10% dogfish liver oil	101.2	101	−0.1	593 ± 291

*Mean values for 16 birds ± the standard deviation.

the serum cholesterol concentration, but in conjunction with corn oil the sterol did produce a significant elevation. Dogfish liver oil was still more effective in this respect than corn oil. As was the case with the mature cockerels the body weight of the laying hens did not alter appreciably during the period of investigation.

The egg production by the hens was influenced by dietary constituents as can be seen from Fig. 1. The incorporation of 1% cholesterol with or without 10% corn oil into the control diet brought about little change in the rate of egg production, but the presence of 10% dogfish liver oil in the cholesterol-containing diet caused a noticeable decrease in production around 5 days and a complete cessation of laying after 7 days on the diet.

Although there was no striking difference in the number of eggs laid by hens on the first three diets mentioned above, the composition of the egg yolks varied considerably (Fig. 2). The incorporation of 1% cholesterol in the diet increased the concentration of egg yolk cholesterol and this effect was enhanced by the presence of 10% corn oil, the value after the 14 days experimental period being twice that in the control eggs. A statistical analysis was performed on the data and Table VII gives the significance obtained for the differences in yolk cholesterol concentration between the diets on individual days. A significant increase in yolk cholesterol of eggs from birds on the cholesterol-containing diet became manifest after 7 days, but this increase was significant after only 5 days when corn oil was also present in the diet. Figure 2 and Table VII do not show the data obtained for eggs from hens fed the dogfish liver oil diet, but during the

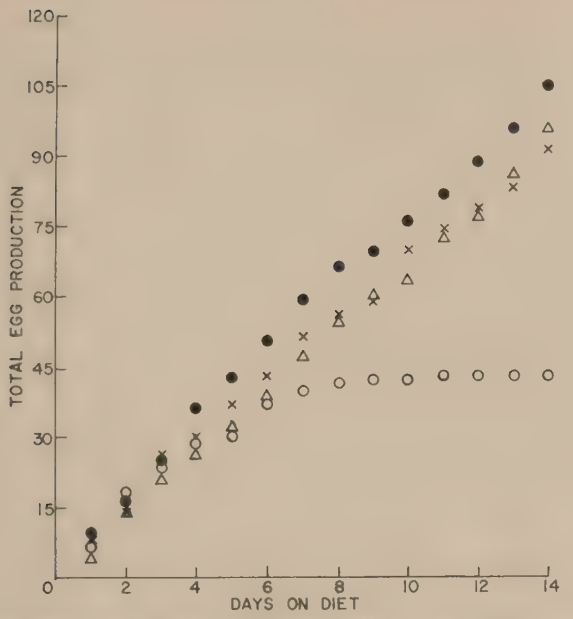

FIG. 1. Egg production of hens on different diets. ● control diet; △ control diet + 1% cholesterol; × control diet + 1% cholesterol + 10% corn oil; ○ control diet + 1% cholesterol + 10% dogfish liver oil.

TABLE VII

Significance of differences between egg yolk cholesterol concentrations

Days on diet	Level of probability at which difference is significant		
	Diets 1 and 2* (%)	Diets 1 and 3 (%)	Diets 2 and 3 (%)
1	N.S.†	N.S.	N.S.
2	N.S.	N.S.	N.S.
3	N.S.	N.S.	N.S.
4	N.S.	N.S.	N.S.
5	N.S.	1	N.S.
6	N.S.	5	N.S.
7	1	1	5
8	1	1	5
9	5	1	5
10	1	1	1
11	1	1	1
12	1	1	1
13	1	1	1
14	1	1	1

*Diets 1, 2, and 3 are the control diet, control diet + 1% cholesterol, and control diet + 1% cholesterol + 10% corn oil, respectively.
†N.S. denotes difference is not significant.

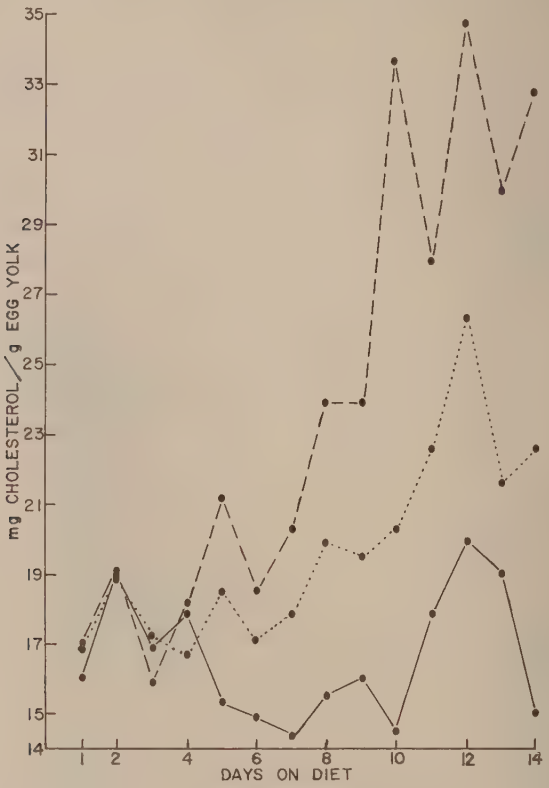

FIG. 2. The effect of diet on cholesterol concentration in egg yolk. —— control diet; . . . control diet + 1% cholesterol; – – – control diet + 1% cholesterol + 10% corn oil.

few days that the birds on this diet laid eggs the egg yolk cholesterol concentration was similar to that in eggs from the control hens. The above results deal with the concentration of cholesterol in the yolks but since the weights of the latter were similar on all diets, the same picture is obtained for the total amount of cholesterol in the eggs.

The unsaponifiable fractions of corn oil and dogfish liver oil were compared with cholesterol and β-sitosterol standards using the chromatographic system described earlier in this paper. Corn oil gave a spot with the same R_f as β-sitosterol and the fish liver oil gave a spot corresponding to cholesterol. No other spots were observed with the material from the oils. Similar studies with the unsaponifiable fractions from the sera of laying hens on the four diets gave in all cases one spot which corresponded to cholesterol. The unsaponifiable material from the yolks of eggs laid on the 14th day of the experiment was examined chromatographically and the results indicated that, regardless of the diet, egg yolks contained cholesterol with no observable contamination of plant sterols.

Discussion

Age and sex appeared to have a strong bearing on the mechanism of cholesterol metabolism in chickens. The normal cholesterol level in young chicks was higher than that in mature birds of the same sex, this decrease with age occurring in both sexes. Moreover, in both young and old birds, males had lower serum cholesterol concentrations than females, an observation which agrees with the work of Lorenz *et al.* (4) which indicated that laying hens had somewhat higher blood total cholesterol values than mature cockerels.

The susceptibility of chickens to hypercholesterolemia induced by dietary cholesterol also varied with the age and sex of the birds. The addition of 1% cholesterol to the control diet brought about an approximately four- to six-fold increase in the serum cholesterol concentration of young pullets and of cockerels of both age groups. On the other hand, the laying hens were more resistant to this type of hypercholesterolemia under the conditions of the present experiment. Hens fed the cholesterol-containing diet for 2 weeks showed no significant increase in the serum cholesterol level but it might well be that a longer period would produce a significant increase. When the diet contained 10% corn oil and 1% cholesterol the serum cholesterol level of the hens increased, but the degree of hypercholesterolemia obtained was still much less than that which occurred in cockerels and young pullets. Since the protein content of the diets was constant, rapidly growing chicks were perhaps suffering a protein lack relative to the situation in laying hens of constant body weight, but it is unlikely that this relative lack was a major cause of the difference in susceptibility to hypercholesterolemia of young chicks and laying hens because mature cockerels of constant body weight were also readily susceptible to dietary-induced cholesterolemia.

The possibility that the presence of plant sterols from ingested corn oil contributed significantly to the observed elevation of serum sterol concentration in corn-oil-fed hens was rendered improbable by the results of the chromatographic analysis which indicated no large amount of plant sterols in the serum. This observation is analogous to the finding of Swell *et al.* (16) that the ingestion of plant sterols by rats did not lead to an accumulation of the sterols in the serum.

The action of dogfish liver oil on cholesterol-induced hypercholesterolemia depended on the age of the birds but not on their sex. In young birds the liver oil reduced the degree of hypercholesterolemia by approximately 50% but complete prevention could be effected by increasing the amount of dietary fish oil (Wood and Topliff (21). In contrast to this behavior, the addition of dogfish liver oil to the cholesterol-containing diet increased the degree of hypercholesterolemia in mature birds. Since the hens stopped laying when the fish oil was present in the diet, it was thought that perhaps the observed increase in serum cholesterol concentration was related to this phenomenon, but such an explanation does not account for the similar action of the oil in mature cockerels. A possible reason for the elevation of serum cholesterol is the presence of cholesterol in dogfish liver oil. The oil used in the experiments con-

tained 1.4% sterol which was shown by chromatographic analysis to be mostly, if not entirely, cholesterol. The amount of "fish oil cholesterol" therefore amounted to 0.14% of the diet. Whether the increase in the amount of dietary cholesterol from 1.00% to 1.14% caused the marked elevation in cholesterol level is open to question. Another possible explanation is that glyceryl ethers present in dogfish liver oil (17) increased the intestinal absorption of cholesterol by reason of their emulsifying properties (18).

Earlier work in this laboratory (2) indicated that the hypocholesterolemic action of fish oils was due in most part to their vitamin A content, and it must be inferred, therefore, from the present results that vitamin A does not possess this action in mature birds. This agrees with the report of Weitzel *et al.* (3) which indicated that vitamin A did not lower serum cholesterol levels in old hens. The vitamin may, however, be responsible for the cessation of egg production observed in the present investigation when dogfish liver oil was incorporated into the diet, since excessive amounts of vitamin A have been shown to affect adversely the reproductive ability of the rat (19) and the mink (20).

The amount of cholesterol in egg yolk was profoundly influenced by the diet of the hens. The presence of 1% cholesterol in the diet increased the egg yolk cholesterol content, an elevation which was enhanced by the incorporation of 10% corn oil into the feed. This result is contrary to the work of Kurnick *et al.* (12), who reported that dietary cholesterol produced no change in egg cholesterol. The difference in the observations may be due to a difference in composition of the experimental diets, especially since the present investigation has shown that the fat content of the cholesterol-containing diet played an important role in the control of egg yolk cholesterol. The experiments reported here were of short duration and longer-term investigations are planned for the near future to ascertain whether the changes in egg yolk cholesterol continue over an extended period.

It is clear from the above results and discussion that laying hens were more resistant to cholesterol-induced hypercholesterolemia than were the other birds, no matter whether the cholesterol was incorporated into the diet alone or together with corn oil. This finding agrees with that of Stamler *et al.* (6) which indicated that a higher dietary cholesterol level was required by laying hens than by roosters to produce the same degree of hypercholesterolemia. One possible explanation is that the hens can eliminate cholesterol via the eggs, a route not available to the other birds. Evidence for this mechanism was brought forth by Stamler's group (6), who showed that the greater resistance of hens to dietary-induced hypercholesterolemia was abolished by oviduct ligation in the birds. The present investigation furnishes further proof by showing that dietary components which caused hypercholesterolemia in chickens also increased the cholesterol content of eggs laid by the hens. If the above proposed mechanism is correct, then substances which cause a cessation of egg production

in cholesterol-fed hens should elevate the serum cholesterol levels. This situation arose in the present studies when 10% dogfish liver oil was included in the diet. The hens stopped laying within 7 days and, in agreement with the proposed theory, a substantial increase in serum cholesterol concentration occurred. The concentration was still lower than that in cockerels fed a comparable diet but this was not unexpected since the hens laid a considerable number of eggs in the first half of the test period and this would doubtless influence the cholesterol level reached after 14 days. However, as pointed out previously, other complicating factors were involved in the action of the dogfish liver oil. The above explanation for the greater resistance of laying hens to hypercholesterolemia cannot be ignored but it is unlikely to be the sole cause since an estrogenic effect on the degree of lipemia and cholesterolemia in chickens has previously been reported (21, 22).

The work reported here has indicated that dietary constituents which increase serum cholesterol levels in hens also increase the cholesterol content of the eggs laid by the birds. It would be interesting if the converse were true and hypocholesterolemic agents decreased egg cholesterol levels, especially since the current trend is to cut down on the human consumption of cholesterol-rich foods.

Acknowledgments

The authors wish to thank Mr. S. A. Haqq for his technical assistance, and Krieger's Hatchery, Ltd., Aldergrove, B.C., for supplying the chicks.

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11-KETOTESTOSTERONE: AN ANDROGEN FOR SOCKEYE SALMON[1]

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Abstract

11-Ketotesterone, a hormone in the blood of sockeye salmon, has been shown to have androgenic activity for this species. It influences skin thickness and coloration, flesh pigmentation, and spermatogenesis in the male. The effect of 11-ketotestosterone is not so pronounced in the female but it influences both skin thickness and coloration. Estradiol increased the mass of the female gonads.

Introduction

An androgen is a substance that promotes the development of secondary sex characteristics. A previous study has demonstrated that 11-ketotestosterone is a potent androgenic hormone as determined by the chick-comb bio-assay (1). This steroid has been isolated, in this laboratory, from the plasma of sexually mature male salmon (2). A substance with the chromatographic mobility of 11-ketotestosterone occurs in high concentration in the plasma of sexually mature female sockeye salmon and is the major adrenocorticosteroid in the plasma of spawned *Salmo salar* males (P.J. Schmidt and D. R. Idler. Submitted for publication).

It was the purpose of the present investigation to determine if 11-ketotestosterone was an androgen for salmon.

The most pronounced secondary sex characteristics of the male sockeye are an intense red coloration of the skin, an elongation of the snout, and the development of a hump on the back. The female undergoes these changes to a much lesser degree than does the male. Less obvious manifestations of increasing sexual maturity are a thickening of the skin and a decrease in the amount of carotenoid pigments (3) in the flesh.

Methods

Several groups of sexually immature sockeye salmon were taken prior to their entrance into Great Central Lake on Vancouver Island during the latter part of June and early July, 1960. These fish were transferred to the outdoor holding ponds of the B.C. Fish and Game Department at Courtenay, B.C., where procedures were developed for holding the fish in good condition in spite of the high water temperatures (4).

The fish used for this experiment were transported to Courtenay on July 10, 1960, and given salt baths, to prevent fungus infection, every 2 days until the experiment was begun on July 20. On July 20, 43 fish were divided into two

[1]Manuscript received June 8, 1961.
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groups and each group was held in an identical tank. The fish from one group (predominantly male) were anaesthetized with MS-222,[3] 44.5 p.p.m. at 9–11° C, and injected intramuscularly in the vicinity of the dorsal fin with 5 mg of 11-ketotestosterone in 0.2 ml of peanut oil. The oil was injected through a No. 18 3-in. needle which had previously been dipped in penicillin G (ca. 1.2×106 units/ml). The control groups were given injections of peanut oil. The injections were made at weekly intervals.

A single fish from the control group was implanted, 5 days after the experiment began, with a pellet containing 37 mg of 11-ketotestosterone and no added carrier. A longitudinal subcutaneous incision of the same width as the pellet was made for a length of about $1\frac{1}{2}$ inches in the side of the fish and instruments were dipped in penicillin to minimize risk of infection. On August 2, 13 days after the beginning of the injection in the initial experiment, four additional fish from the control group were started on injections of 11-ketotestosterone. These four fish and the fish given the pellet were marked with yellow tags. On the same date five of the control group (four females and one male) were injected with estradiol and these injections were continued at weekly intervals (5 mg/0.2 ml of peanut oil). These fish were marked with red tags.

The tanks were checked at frequent intervals and dead fish were removed immediately. Post-mortem storage experiments carried out in this laboratory have demonstrated that flesh color is not significantly affected by the short post-mortem storage encountered in these experiments.

Flesh color was measured on freshly cut steaks and this and the other determinations were made on a steak taken from the same location in each fish. Fat and moisture determinations were performed on the flesh after canning (5).

Results and Discussion

At the end of 2 weeks there was a very significant visual difference in the appearance between the group injected intramuscularly with 11-ketotestosterone and the control group. A photographic record was obtained after the experiment had been in progress for 19 days. The fish in the control group still had the silvery appearance of "green" fish, while all the male fish which had been injected were colored an intense red. On August 17, when the experiment had been in progress for 28 days, further pictures were obtained. The fish in the control group still had their silvery appearance, while the males which had been injected were already beginning to show a darkening of their skin which a week before had been bright red. The injected fish were also beginning to show a pronounced elongation of the snout. At this time it was difficult to distinguish the males from the females in the control group but post-mortem examination (Table I) showed that there were males in the control group (blue tags and no tag).

By the time the experiment had been in progress for 28 days it became apparent that there was no observable difference in the appearance of the control

[3]Tricaine methanesulphonate available from Sandoz Pharmaceuticals, Montreal, Que.

TABLE I

Effect of 11-ketotestosterone and estradiol on salmon

No. or tag	Sex	% moisture	% fat	Gonads (% live wt. of fish)	Color reading (*a*)	Skin thickness (mm)	Death	Egg size (mm)
Green, group I*								
8	M	78.8	2.63	0.37	0.6	.762	Sept. 7(K)	
6	M	79.2	2.59	1.08	4.3	.762	Sept. 7(K)	
7	M	78.8	2.63	1.00	4.2	.838	Sept. 7(K)	
1	F	76.4	4.35	5.90	2.9	.711	Sept. 7(K)	3.5–4.0
21	M	81.0	2.92	0.68	2.4	.965	Sept. 19(D)	
Green, group II†								
23	M	76.0	3.07	0.88	10.8	.686	Aug. 19(D)	
4	F	77.6	3.69	5.03	7.6	.737	Sept. 7(K)	
12	M	76.2	3.29	0.41	5.9	.940	Sept. 7(K)	
11	M	76.2	3.29	0.58	8.0	.864	Sept. 7(K)	
9	F	77.6	3.69	7.80	3.3	.686	Sept. 7(K)	4.0–4.5
3	M	78.4	2.33	0.60	5.5	.864	Sept. 7(K)	
24	F	77.0	2.78	5.86	26.1	.660	Aug. 25(D)	4.5
25	F	73.6	5.55	4.48	27.4	.711	Aug. 25(D)	4.0
28	M	74.0	4.34	0.18	18.2	.711	Aug. 23(D)	
37	F	75.6	5.30	10.23	6.2	.864	Sept. 6(D)	4.5–5.0
38	M	—	—	0.51	1.4	.737	Sept. 29(D)	
Red tag*								
22	F	74.0	4.87	12.21	32.4	.508	Aug. 19(D)	
26	F	77.0	2.60	13.93	32.0	.533	Aug. 22(D)	5.0
34	M	72.8	2.99	0.28	27.2	.559	Aug. 24(K)	
2	F	76.6	2.70	20.70	25.7	.533	Sept. 7(K)	6.0
20	F	81.0	2.06	28.10	9.8	.610	Sept. 17(D)	5.5–6.0
Yellow tag*								
35	M	75.8	2.21	0.92	11.7	.914	Aug. 24(D)	
36	M	74.6	2.98	1.60	23.6	.635	Aug. 24(D)	
5‡	F	77.3	3.31	6.70	5.0	.762	Sept. 7(K)	4.0–4.5
10	F	74.2	5.86	5.66	11.8	.787	Sept. 7(K)	4.0
19	F	79.0	3.05	11.70	4.8	.864	Sept. 11(D)	5.5–6.0
Blue tag*								
15	M	73.0	4.91	2.80	35.8	.610	Sept. 7(K)	
27	F	74.0	3.33	10.28	30.4	.610	Aug. 22(D)	5.0–5.5
34	F	73.6	3.16	8.12	34.2	.685	Aug. 23(K)	4.0–4.5
No tag*								
29	F	74.2	2.22	11.26	29.0	.508	Aug. 23(K)	4.5–5.0
32	F	73.6	5.79	8.90	27.6	.559	Aug. 23(K)	4.0
13	F	71.6	6.25	6.00	35.6	.635	Sept. 7(K)	3.5
14	M	73.0	4.91	1.70	37.0	.635	Sept. 7(K)	
16	F	71.6	6.25	9.00	36.1	.432	Sept. 7(K)	4.0
17	F	71.6	4.90	6.30	35.8	.584	Sept. 7(K)	3.8
18	F	72.6	4.28	8.50	39.9	.584	Sept. 7(K)	4.0–4.5
30	M	73.8	2.90	3.00	32.3	.457	Aug. 23(K)	
31	M	72.0	2.52	2.07	27.0	.584	Aug. 23(K)	
39	F	—	—	12.00	28.3	.508	Oct. 25(D)	4.5–5.0
40	F	—	—	19.00	—	—	Dec. 15(D)	4.5
41	F	—	—	14.00	—	—	Dec. 15(D)	4.0–4.5
42	F	—	—	12.60	—	—	Jan. 2(D)	4.0–4.5
43	M	—	—	2.03	—	—	Jan. 13(D)	

NOTE: K, killed; D, died.

*Green tag group I fish given 11-ketotestosterone injections weekly from July 20 until death. Red tag fish injected with estradiol from August 2 until death. Blue tag fish injected with peanut oil only from July 20 until death. Yellow tag fish, except for No. 5, were given 11 ketotestosterone injections weekly beginning August 2. No tag fish injected with peanut oil only from July 20 until August 17.

†11-Ketotestosterone injections stopped August 17.

‡A 37-mg pellet of 11-ketotestosterone implanted in this fish.

fish which had been receiving peanut oil and those which were not being injected. These fish were continued on peanut oil injection until they died or were killed and these were given a blue tag. For the remainder the peanut oil injections were discontinued.

The fish were moved from Courtenay, B.C., to the Vancouver Public Aquarium on August 17 (28 days after the start of 11-ketotestosterone injections).

On August 17 (15 days after the beginning of the estrogen injections) the female estrogen-injected fish had a much darker coloration in the belly region than did the controls, and they had also developed a yellow coloration particularly in the peduncle area. The male fish which were given their first injection on August 2 and the one fish which received a pellet (yellow tag) showed well-developed secondary sex characteristics and the lone female (yellow tag) had a moderate red coloration of the skin and a slight yellowing around the anus. At this time two of the fish, later shown by autopsy to be a male and female, in the control group showed a red coloration of the skin. However, it was not so intense as the coloration of the 11-ketotestosterone injected fish several weeks earlier and it was not present to any significant extent on the underside of the fish.

After August 17, injections of 11-ketotestosterone were discontinued except to a small group composed of four males and one female (green tag, group I, Table I).

The observations made in the field and a study of the color photographs which were taken clearly showed that 11-ketotestosterone was an androgenic substance for salmon. However, it was considered desirable to attempt to obtain some quantitative expression of this fact. It was felt that flesh color, skin thickness, and gonad development might be suitable criteria. However, it was necessary to sacrifice the fish in order to obtain this information. The problem was further complicated by the fact that some of the fish died from time to time and it was difficult to sacrifice sufficient numbers at any one time to be sure that the results would be significant. Fortunately, the differences between the injected and the control fish of both sexes were of a sufficiently large order of magnitude and sufficiently consistent that certain conclusions can be drawn. The data obtained for fish sacrificed on September 6 and 7 are shown in Table II. In a previous study carried out in this laboratory it has been shown that the color of the flesh expressed as the "*a*" value on the Gardner Automatic Color Difference Meter[4] is a good quantitative measure of the red pigmentation of the flesh (6). The flesh of prime quality immature sockeye generally gives an "*a*" value in excess of 30. Both male and female fish in the control group had "*a*" values over 30 and by contrast all 11-ketotestosterone injected fish had "*a*" values under 10. Flesh with an "*a*" value of less than 10 has at the most only traces of red pigment. The three male fish which received their last injection of 11-ketotestosterone on August 17 (3 weeks before death)

[4]Manufactured by Gardner Laboratory Inc., Bethesda 14, Maryland, U.S.A.

TABLE II

Effect of 11-ketotestosterone on sockeye sacrificed September 7

Rx	Sex	Fish	Flesh color "a"*	Skin (mm)	Gonads† (%)	Sperm‡
Steroid	M	6§	0.6–8.0	0.76–0.94	0.37–1.08	+
None	M	2	36, 37	0.61–0.64	1.7 –2.8	–
Steroid	F	4‖	2.9–7.6	0.69–0.86	5–10	
None	F	4	36, 40	0.43–0.63	6–9	

*Value by Hunter Color Difference Meter.
†Gonads expressed as percentage of the live weight of the fish.
‡Microscopic examination of spermatic fluid.
§Last injection of three fish on August 17.
‖One fish sacrificed September 6. Last injection of fish on August 17.

had an average flesh "*a*" value of 3.0 as compared with 6.5 for the three males which received 11-ketotestosterone until 1 week before death. All male fish which continued to receive injections had lower flesh "*a*" values than those for which the injections were stopped (Table I). The male fish sacrificed on August 19–25 also showed much lower flesh "*a*" values for the 11-ketotestosterone-injected than for the control. While there appeared to be a slight difference at this time between the color of the flesh of injected and control females, it was not nearly so pronounced as it was with the fish sacrificed on September 7.

Both male and female 11-ketotestosterone-injected fish sacrificed on September 7 had thicker skins than those of the controls. The same relationship also held for males sacrificed on October 19–25 but, as was the case with color, the effect on females was not so great for those fish sacrificed on the earlier dates.

The ratio of the weight of the gonads to the live weight of the fish has been shown to be a sensitive indicator of sexual maturity in investigations carried out on the Fraser River sockeye (5). This expression of maturity was particularly useful in determining the sexual maturity of females. The gonads of the 11-ketotestosterone-injected males were smaller than were the controls for fish sacrificed on September 7 (Table II). This was also true for males sacrificed August 19–25 (Table III). There was no discernible difference in the size of the gonads of injected or control females sacrificed on September 7. However, only one fish received 11-ketotestosterone injections after August 17. There was a difference in the size of the gonads of females sacrificed on August 19–25,

TABLE III

Effect of 11-ketotestosterone on sockeye sacrificed August 19–25*

Rx	Sex	Fish	Flesh color "a"	Skin (mm)	Gonads (%)
Steroid	M	2	10.8, 18.2	.69, .71	.18, .88
None	M	2	27.0, 32.3	.46, .58	2.07, 3.0
Steroid	F	2	26.1, 27.4	.66, .71	4.5, 5.9
None	F	4	27.6, 34.2	.51, .69	8.1, 11.3

*Male and female control fish sacrificed August 23. One male injected fish died on August 19 and the other August 23. Three female controls were killed August 23 and one died August 22.

with the gonads of 11-ketotestosterone-injected females being smaller than those of the control fish. The data for females are not as conclusive as those for males.

The testes of males sacrificed on September 7 contained the milky fluid characteristic of sexually mature fish and microscopic examination showed that sperm were present. There was no significant amount of spermatic fluid in the testes of control fish sacrificed up until September 7 but it was present in the steroid-injected males sacrificed on August 19–25.

There also appeared to be some correlation between the color of the liver of the fish at death and the treatment which the fish had received. With the exception of a single male, all fish which were injected with 11-ketotestosterone varied in color from green brown to dark green. Livers of the control fish were all brown with the exception of two females which were green and brown. The estrogen-injected females also had brown livers.

The data for the fish receiving the pellet of 11-ketotestosterone and injections which did not begin until August 2 (yellow tag, Table I) confirm that females injected with 11-ketotestosterone and sacrificed September 7 and 11 had very little color in their flesh. It therefore seems reasonable to conclude that the loss of color from the female flesh occurred to the greatest extent between August 17 and September 7.

Three females which died on December 25, 1960, or January 2, 1961, had gonads which averaged 15.2% of the live weight of the fish and the eggs were 4.0 -4.5 mm in diameter. Another female which died on October 25 had gonads which were 12.0% of the live weight of the fish and the eggs were 4.5–5.0 mm. The gonads of six females killed on August 23 or September 7 averaged 8.3% of the live weight of the fish for the controls and the eggs were approximately 4 mm in diameter. The effect of estradiol injections on the size of the gonads was already apparent by August 19–22 when the gonads of two hormone-treated fish averaged 13.1% of the live weight. However, the estrogen-injected females sacrificed on September 7 and 17 had gonads which weighed 21 and 28%, respectively, of the live weight of the fish. These latter fish had eggs which were 5.5 -6.0 mm in diameter. The more advanced maturity of the 11-ketotestosterone-injected fish was, on the average, also reflected in their high body water and low body fat content relative to the control group (Table I) (6).

Acknowledgment

Mr. A. P. Ronald assisted in the preparation phase of this investigation.

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SOME FEATURES OF THE UPPER ZONE OF THE SUB-ARCTIC PACIFIC OCEAN

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ABSTRACT

The upper zone is defined by the depth at "D" of the homogeneous (isohaline and isothermal) layer at the end of the cooling season (March-April). The limit "D" is defined in the logarithmic plot of the salinity structure by a marked discontinuity at the top of the halocline. The temperature and salinity at this limit persist beneath the thermocline and secondary halocline which appear in summer. They are modified slightly by entrainment and transport. These conclusions are supported by comparison of winter and summer data from the eastern sub-Arctic. Therefore it is possible, from an oceanographic survey made in the summer, to identify the extremes of temperature that existed throughout the depth (D) of the upper zone, throughout the whole region, during the previous winter, for the periods studied.

This analysis is applied to four years of summer data (1956 through 1959) and significant variations in the winter temperatures and their distribution are shown.

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INTRODUCTION

It has been confirmed that the salinity structure, with minor variations, is a permanent feature of the eastern sub-Arctic[1] (Dodimead, 1958). An examination of extensive data in this region showed that this structure could be accurately delineated by plotting salinity as a function of the logarithm of the depth (Doe, 1955; Tully and Dodimead, 1957). Such a plot (Fig. 1) leads to a definition of 3 distinct zones; an upper zone of relatively homogeneous, low salinity water, a transition zone characterized by a marked halocline, and a lower zone in which the salinity increases gradually with depth (Tully, 1957). These are separated by two marked discontinuities at the upper and lower limit of the halocline. They have been designated "D" and "L" respectively, as shown in the figure.

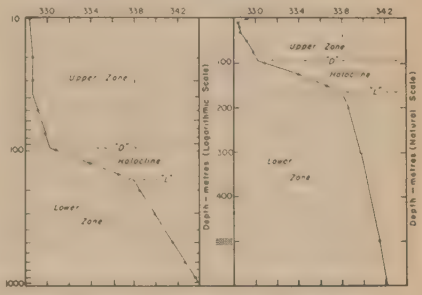

FIGURE 1.

Recently it has been reported that the features of an estuarine system, the "entrainment" process and fresh water mechanism occur in the sub-Arctic region (Tully and Barber, 1959). In the report the significance of, and the variations at "L" (bottom of halocline) were discussed. The significance of the upper limit "D" was discussed briefly. It is now possible to propose a mechanism for the formation of the upper zone and the significance of the depth, salinity and temperature at "D".

Received for publication April 7, 1960. Original, English.
Source: Contribution from the Fisheries Research Board of Canada, Pacific Oceanographic Group, Nanaimo, B. C. INPFC Document No. 358.
Bulletin 3, Int. North Pac. Fish. Comm., January, 1961.

1. Sub-Arctic region can generally be characterized by the presence of an upper zone and halocline in the salinity structure. The region includes the transition zone as defined in "The Oceans" (Sverdrup *et al*) which is bounded on the south by the sub-Tropic waters.

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FRB No. 669

SALINITY STRUCTURES IN THE NORTH PACIFIC OCEAN

In Figure 2(b) (logarithmic plot) and Figure 2(c) (natural plot) are shown representative salinity structures in the North Pacific Ocean (positions indicated in Figure 2(a)). It is apparent that the upper zone and hence the limit "D" can always be defined, and that the salinity structure can be interpreted logarithmically within the sub-Arctic region.

As shown in Figure 2(b), the lower limit "L" is not an invariable feature of the sub-Arctic region. In areas of mixing, such as the vicinity of the Aleutian Islands and in some areas of the Bering Sea, the marked halocline deepens (Fig. 2(b), K), and the lower limit "L" becomes indefinite (Fig. 2(b), J). This latter feature has been reported previously (Dodimead, 1958) and has been demonstrated in the Strait of Juan de Fuca by Tully (1957). In extreme cases, the structure becomes nearly isohaline throughout the column (Fig. 2(b), L).

Within the transition zone, the upper zone and halocline become less pronounced (Fig. 2(c)). They both disappear in the sub-Tropic waters (Fig. 2(b), G; Fig. 2(c)).

It is noted that in the western sub-Arctic, when the lower limit of the halocline can be defined, the salinity at "L" approximates 33.8‰. This is very near the values that have been reported for the eastern sub-Arctic (Doe, 1955; Tully and Dodimead, 1957; Dodimead, 1958.)

SALINITY AND TEMPERATURE STRUCTURES AT OCEAN STATION "P"

A sequence of salinity and temperature structures (natural plot) at Ocean Station "P" (lat 50°N; long 145°W) is shown in Figure 3. In the following discussion it must be noted that the movement of water in the vicinity of Ocean Station "P" is generally slow, 1–2 miles per day. In addition the water reaching this area is subject to similar climatic conditions as that at Ocean Station "P" and can be regarded as having resided there.

In Figure 3 are shown the changes that occur in the structures from the end of the cooling period, March, through the period of heating, April to October (Tabata, 1958), and to the commencement of the next heating period. It is seen that the salinity and temperature structures are isohaline and isothermal respectively to "D" during the late cooling period. Both the salinity and temperature structures undergo changes above "D" during the period of heating. The structures again become homogeneous during the following cooling period. These changes recur from year to year. It is apparent that the upper zone is established during the period of cooling, and although it undergoes changes, it can be defined throughout the period of heating and is again re-established during the winter period. The salinity and temperature established from the surface to "D" (upper zone) will depend on the degree of cooling, strength of winds, amount of fresh water in the upper zone, and to some degree on the temperature which the water in the upper zone attained during the previous summer. It is evident that these processes do not extend below "D".

Three noticeable changes occur in the salinity structure during the period of heating. These can be related to the development of a marked thermocline within the upper zone (Fig. 3(c)) and the "entrainment" process (Tully and Barber, 1959).

First, because of the stability inherent in the thermocline, which reaches a maximum in early September, the extent of mixing under extremes of summer winds is limited. It is defined by the relatively isohaline and isothermal layer, which is generally between 10 and 30 metres in depth.

Secondly, coincident with the thermocline, a secondary halocline may develop. Fresh water added to the upper zone in summer is conserved in the region above the thermocline.

Thirdly, below the thermocline, and the secondary halocline, if present, the salinity structure, instead of being isohaline, as during the period of cooling, develops a slight gradient to "D". This change in structure can be related to the "entrainment" from the halocline, of water of higher salinity than that immediately above it (Tully and Barber, 1959). This upward transfer from the halocline is probably limited to some level within the thermocline.

In Figure 4 are shown the early stages of reestablishment of the upper zone. During the period, October through November, the thermocline deepens and is partially destroyed by mixing. At the same time the secondary halocline deepens, and it is difficult to differentiate the secondary and primary halocline (Fig. 4(b), (c)). As a result a relatively shallow isohaline and isothermal upper zone is formed. As the season advances both the top of the halocline and thermocline deepen. This process continues through the cooling period until the waters become homogeneous to a depth defined in the salinity struc-

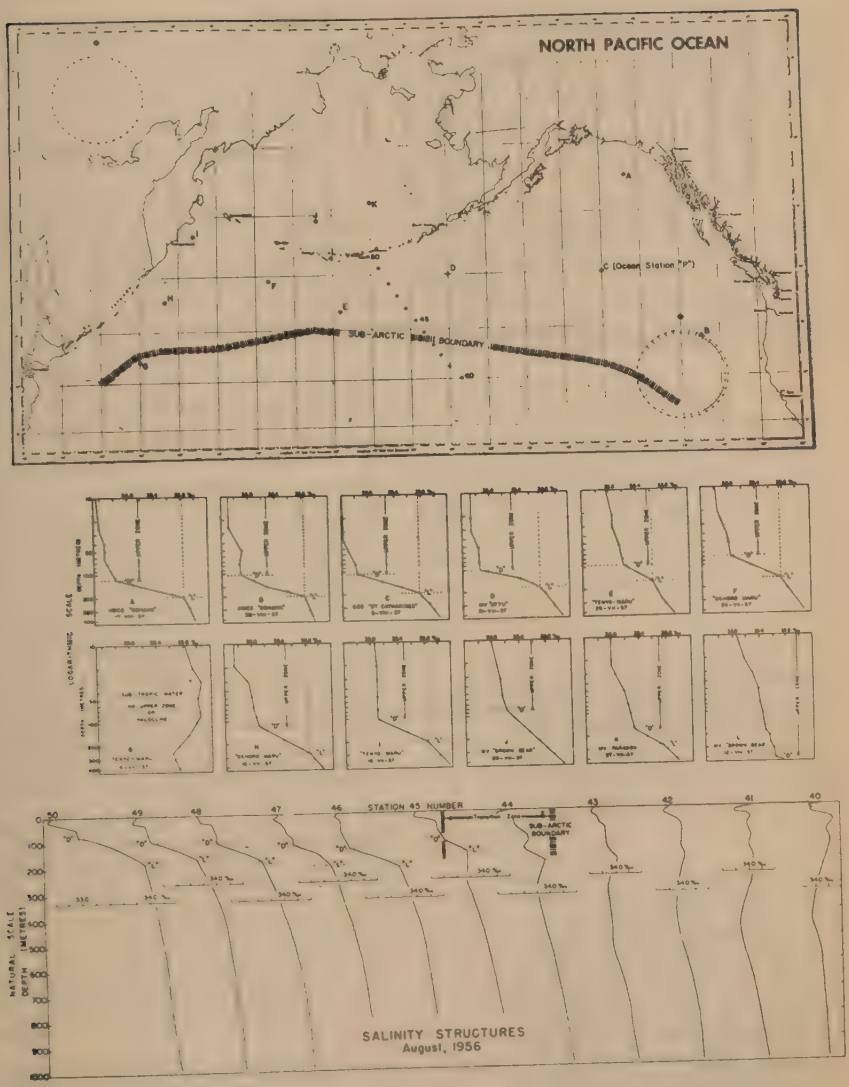
NORTH PACIFIC OCEAN
SUB-ARCTIC BOUNDARY
C (Ocean Station "P")
STATION 45 NUMBER
SUB-ARCTIC BOUNDARY
NATURAL SCALE DEPTH (METRES)
SALINITY STRUCTURES
August, 1956

FIGURE 2. a, b, c

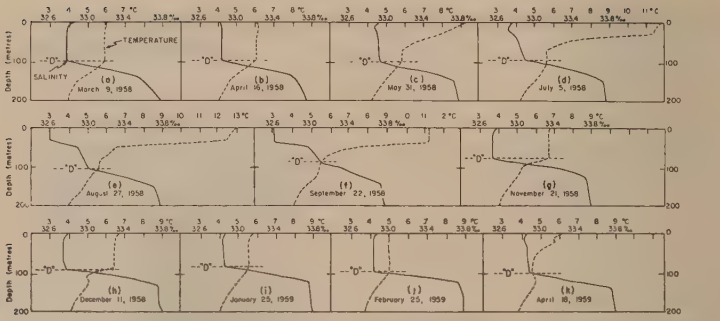

FIGURE 3. Salinity (–) and temperature (– –) structures at Ocean Station "P" (lat 50°N, long 145°W) March 1958–April 1959.

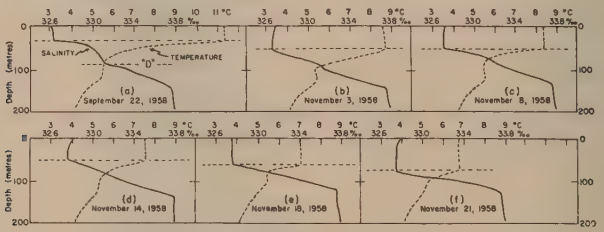

FIGURE 4.

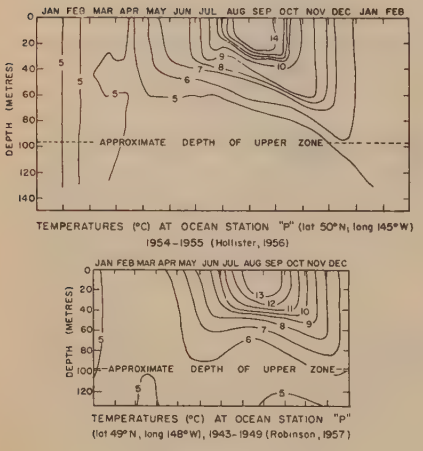
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB
DEPTH (METRES)
APPROXIMATE DEPTH OF UPPER ZONE
TEMPERATURES (°C) AT OCEAN STATION "P" (lat 50°N, long 145°W)
1954–1955 (Hollister, 1956)
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
DEPTH (METRES)
APPROXIMATE DEPTH OF UPPER ZONE
TEMPERATURES (°C) AT OCEAN STATION "P"
(lat 49°N, long 148°W), 1943–1949 (Robinson, 1957)

FIGURE 5.

ture by the point "D" (Fig. 3(j)).

During the early period of heating and especially during the early period of cooling, warm water penetrates to greater depths within the upper zone than during mid-summer. This feature is shown in Figure 5 (Hollister, 1956; Robinson, 1957). The latter period is one of maximum winds, the former being one of moderate winds (Tabata, 1959). Because of the penetration of warm water to moderate depths in the upper zone, it is suggested that the mixing to homogeneity and the establishment of the depth "D" must be due primarily to wind action.

Further evidence in support of wind action is found in the depth of the upper zone calculated from Ekman's emperical relation

$$D = \frac{7.6\,W}{\sqrt{\sin\varphi}} \quad \text{(Sverdrup } et\ al\text{, 1942)}$$

This expression gives a value of 112 metres for wind speeds of 25 knots which are average in the region during the cooling period (Tabata, 1959). The calculated value is near that observed at Ocean Station "P" (95–105 metres).

Under more extreme conditions of winds, the homogeneous zone would probably deepen to some greater depth where the stability inherent in the halocline would retard further deepening of the upper zone.

TEMPERATURE STRUCTURES

It has been shown that the upper zone is established during the period of cooling and that the temperature structure is isothermal in this zone. By the end of the cooling period, two extremes of temperature structure are found in the sub-Arctic region; an isothermal layer to "D" and a negative thermocline (Fig. 3); and an isothermal layer to "D" and a positive thermocline (Fig. 6(a)). In either case the winter thermoclines lie in the halocline. Between these extremes the water column may be nearly isothermal throughout the upper zone and halocline. These structures are primarily dependent upon the extent of cooling and the temperature of the water in the halocline.

As a result of surface heating and the development of a thermocline within the upper zone in summer, two primary temperature structures are found. The first are those shown in Figure 3 for the summer months. The second has a subsurface minimum at "D" and a maximum in the halocline (Fig. 6(b)). This structure is very common in the western and to a lesser extent in the eastern sub-Arctic. The subsurface minimum has been reported as "dichothermal" water by Uda (1935) who considered it to be formed by cooling during the previous winter. It has not been associated with the depth "D" defined in the salinity structure. It is now evident that the temperatures of the water at "D" extended to the surface during the winter months.

The upward mixing processes, "entrainment", that change the salinity structure in the vicinity of "D" during the summer months will alter the temperature structure slightly. In the case of the "dichothermal" structure, temperatures of the waters at "D" in summer will probably be slightly higher than those established in winter, since the waters above and below "D" are warmer than those at "D". In the other structure, the temperature established at "D" in winter will probably be similar to that observed in the summer, since the temperature gradient in the vicinity of "D" is generally small.

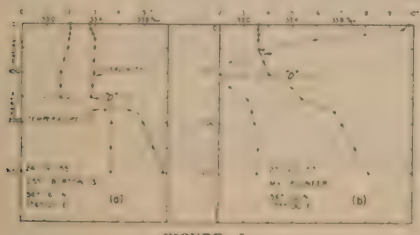

FIGURE 6.

It is now apparent that the extremes of temperature and salinity of the water established in the homogeneous upper zone during the winter persist within small limits and can be recognized at the depth "D" through the following spring and summer. It is further proposed that the winter distributions can be recognized in the summer data. This is discussed in the following sections.

SUMMARY OF DATA

The results of the analysis from the salinity *vs.* logarithm of the depth plot are shown in Figures 7 through 12 for 1956, 1957, 1958 and 1959. The summer distributions were obtained using mainly July-August data, made available by the oceanographic and fisheries agencies in Japan (12, a, b), United States (13, a, b, c), and Canada (14). The only winter data available were from the eastern sub-Arctic. In some areas, where salinity data were lacking, or the depth intervals of the observations were not sufficient to define the structure, the temperature at "D" was determined from the temperature structure alone if a marked "dichothermal" structure existed. This method is not always possible, since in some regions, especially the eastern sub-Arctic, small inversions of temperature occur which are not a result of previous winter cooling, but may be a remnant from cooling two winters previous, or due to lateral intrusion of water in or below the halocline.

DEPTH OF UPPER ZONE

The depth of the upper zone is shown in Figures 7 and 8. Over the eastern sub-Arctic (east of longitude 160°W), the average depth was of the order of 90 to 95 metres, with little evidence of seasonal variation from winter to summer. In the Alaska Gyral, "D" was generally found at depths less than 75 metres and is associated with the dome (anticline of lower zone water, Tully and Dodimead, 1957) and the anticlockwise circulation in this region. Near the Canadian coast and southward the depth at "D" was generally less than 75 metres. This is a region of relatively warm brackish water. In

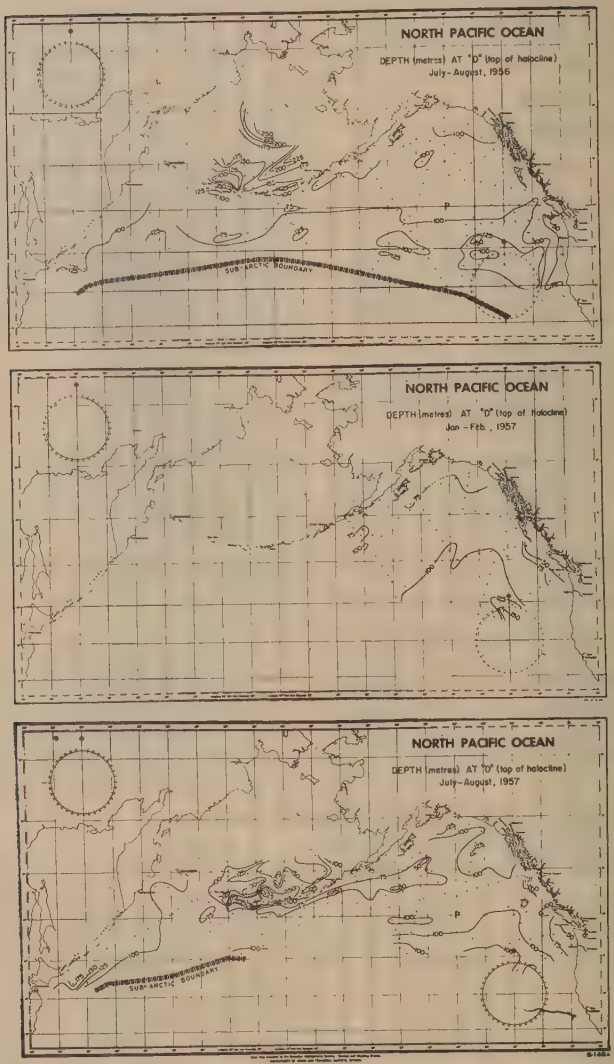
NORTH PACIFIC OCEAN
DEPTH (metres) AT "D" (top of halocline)
July - August, 1956
SUB-ARCTIC BOUNDARY
NORTH PACIFIC OCEAN
DEPTH (metres) AT "D" (top of halocline)
Jan - Feb., 1957
NORTH PACIFIC OCEAN
DEPTH (metres) AT "D" (top of halocline)
July - August, 1957
SUB-ARCTIC BOUNDARY

FIGURE 7.

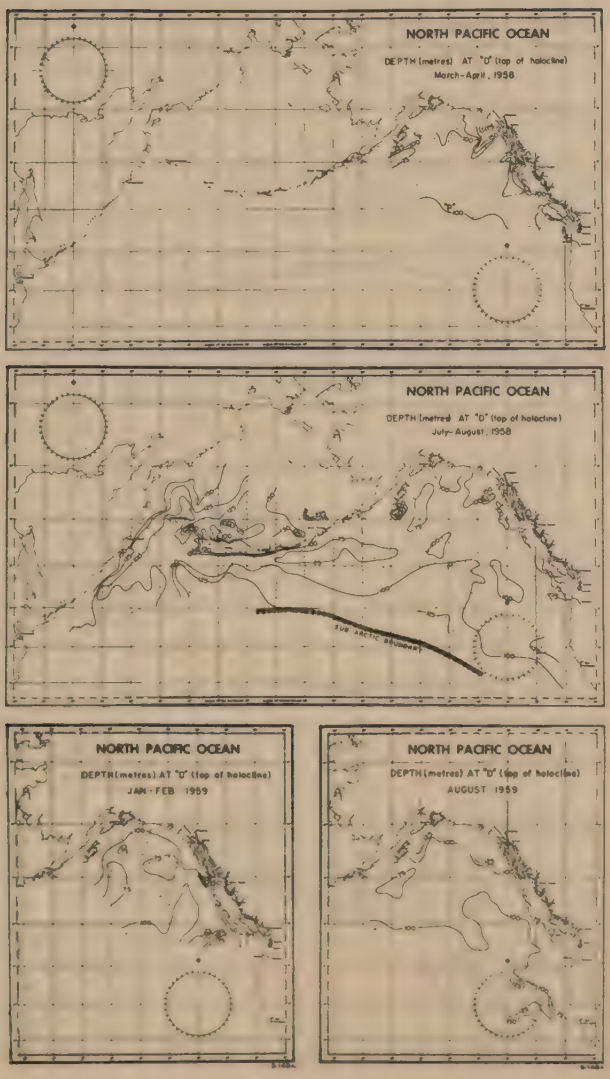
NORTH PACIFIC OCEAN
DEPTH (metres) AT "D" (top of halocline)
March - April, 1958
NORTH PACIFIC OCEAN
DEPTH (metres) AT "D" (top of halocline)
July - August, 1958
Sub ARCTIC Boundary
NORTH PACIFIC OCEAN
DEPTH (metres) AT "D" (top of halocline)
JAN - FEB 1959
NORTH PACIFIC OCEAN
DEPTH (metres) AT "D" (top of halocline)
AUGUST 1959

FIGURE 8.

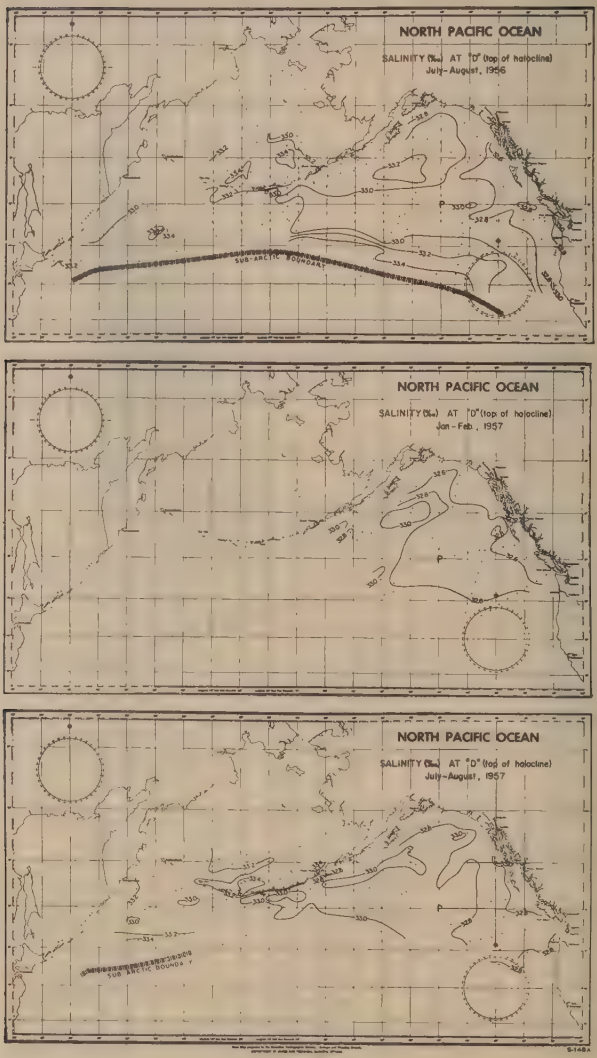
NORTH PACIFIC OCEAN
SALINITY (‰) AT "0" (top of halocline)
July–August, 1956
SUB-ARCTIC BOUNDARY
NORTH PACIFIC OCEAN
SALINITY (‰) AT "0" (top of halocline)
Jan–Feb., 1957
NORTH PACIFIC OCEAN
SALINITY (‰) AT "0" (top of halocline)
July–August, 1957
SUB-ARCTIC BOUNDARY

FIGURE 9.

NORTH PACIFIC OCEAN
SALINITY (‰) AT "D" (top of halocline)
March-April, 1958
NORTH PACIFIC OCEAN
SALINITY (‰) AT "D" (top of halocline)
July-August, 1958
NORTH PACIFIC OCEAN
SALINITY (‰) AT "D" (top of halocline)
JAN.-FEB. 1959
NORTH PACIFIC OCEAN
SALINITY (‰) AT "D" (top of halocline)
AUGUST 1959

FIGURE 10.

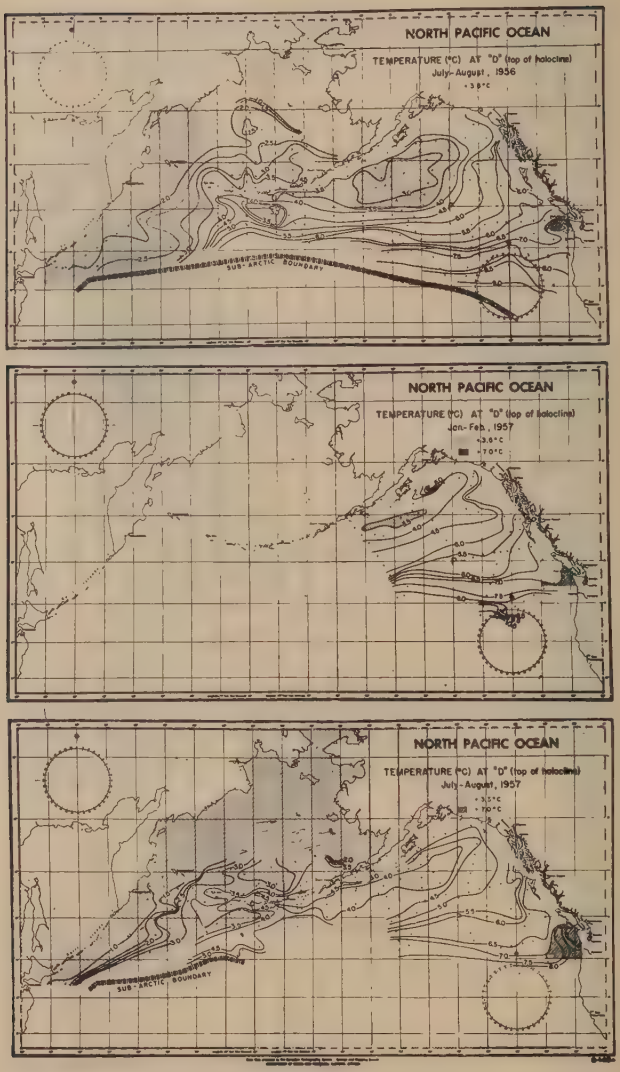
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
July - August, 1956
SUB - ARCTIC BOUNDARY
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
Jan.- Feb., 1957
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
July - August, 1957
SUB - ARCTIC BOUNDARY

FIGURE 11.

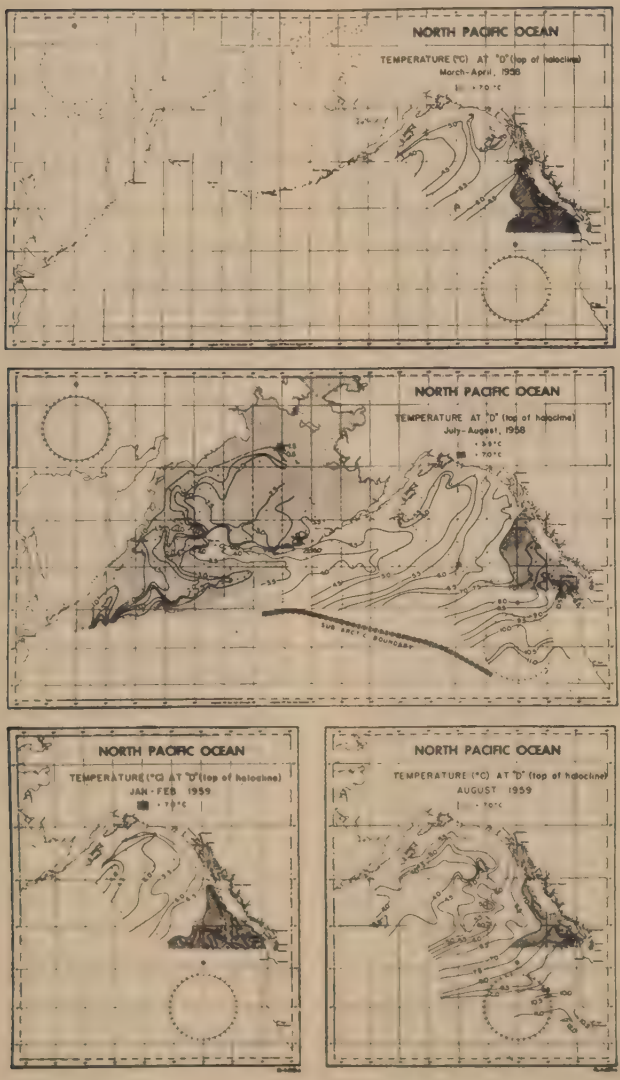
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
March-April, 1958
NORTH PACIFIC OCEAN
TEMPERATURE AT "D" (top of halocline)
July-August, 1958
SUB ARCTIC BOUNDARY
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
JAN-FEB 1959
NORTH PACIFIC OCEAN
TEMPERATURE (°C) AT "D" (top of halocline)
AUGUST 1959

FIGURE 12.

the northeastern part of the Gulf of Alaska, the upper zone was generally deeper than 100 metres.

The largest variations in the depth of the upper zone were found in the vicinity of the Aleutian Islands and in the Bering Sea. These are areas of marked vertical mixing. Near the Asian coast, the upper zone was relatively deep. This is an area of extreme cooling. In other areas of extreme cooling (eastern and northern Bering Sea), the stability of the upper zone, due to fresh water influence, limits the depth of vertical mixing.

SALINITY DISTRIBUTIONS AT "D"

The salinity distributions at "D" are shown in Figures 9 and 10. The highest salinity water was found in the vicinity of the Aleutian Islands, the Bering Sea and in the transition zone (33.2–33.4‰). Relatively high salinity water was present in the Alaska Gyral (33.0–33.2‰). As expected, there appears to be some seasonal variation (winter to summer) in the salinity at "D", higher values occurring during the summer than during the previous winter. The variations were generally less than 0.2‰ within a locality. Yearly variations were of the same order of magnitude. The most marked changes occurred during 1958. In the Gulf of Alaska, salinities at "D" were lower than those observed in the previous periods. At the same time in the Bering Sea salinities were higher than previously.

TEMPERATURE DISTRIBUTIONS AT "D"

Temperature distributions (Figs. 11 and 12) are most interesting because of the marked year to year variations that have occurred. The stippled areas represent water of temperatures less than 3.5°C. In August, 1956, cold water (less than 3.0°C) was present in the Alaska Gyral. After this period the water, generally, became progressively warmer to the summer of 1958. The disappearance of cold water from this area was accompanied by an increase in the temperatures of the waters between Ocean Station "P" and the coast, and northward into the eastern part of the Gulf of Alaska. This is indicated by the crosshatched areas of water greater than 7.0°C. This phenomenon is not solely associated with the degree of cooling and heating since it has been shown that an intrusion of warm water below the depth of seasonal influence occurred during these periods (Tully *et al,* 1959). Following the summer of 1958, the waters in these areas have become colder at "D". This suggests a dissipation or a retreat of the warm water intrusion.

South of the Aleutian Islands, the water was relatively warm (warmer than 3.5°C) during the summer 1956, except for a small relatively cold cell in the vicinity of 180° longitude, and the extension of the tongue of cold water from the Alaska Gyral. A slight change is noted in the summer 1957. The main body of cold water appears to have intruded further east than previously. A marked change is noted in the summer 1958. The waters were generally cold (colder than 3.5°C) further eastward and southward in this region than in the two previous periods. Thus it is seen that when relatively warm conditions appeared in the eastern sub-Arctic, relatively cold conditions prevailed in the western sub-Arctic.

It is noted that the temperature distributions at "D" in the eastern sub-Arctic were generally the same from winter to the following summer, except in the Alaska Gyral, for the periods 1956 and 1958 (Figs. 11 and 12). In this area, the water at "D" was slightly warmer in summer (0.2 to 0.5°C) than in the previous winter. This warming can be attributed to mixing resulting from "entrainment", as this is generally an area of dichothermal structure.

To casual inspection it appears that the summer distribution of temperature at "D" is not as representative of the actual winter distribution at "D" for 1959 (Fig. 12) as was the case for the previous periods.

The discrepancy between these two periods can be explained. First, there was fairly marked cooling after the completion of the winter survey (January 1959). This was not the case in the other winter surveys (Fig. 13). Thus the

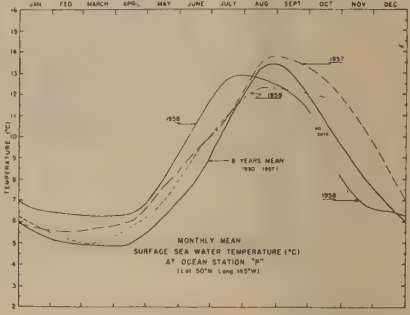

FIGURE 13.

minimum of the winter conditions was not observed. Secondly, it is suggested that there has been a change in the general trend of warming which has occurred from 1955 through 1958. This is discussed by Tully *et al* (1959). It is associated with the change in the circulation in this region. Thus it appears that the circulation was returning to the situation observed during 1956. These two factors can account for the differences observed.

CONCLUSIONS

It can be concluded that for the eastern sub-Arctic, the temperature and salinity distributions at "D" during the summer are indicative of those during the previous winter, providing minimum winter conditions were observed. It follows that each summer distribution reflects very closely that of the previous winter for the whole of the upper zone. It is proposed that these conclusions are applicable for the whole of the sub-Arctic region for these periods.

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USE OF PRESERVATIVES AND ANTIBIOTICS IN THE PRESERVATION OF FRESH FISH

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In 1939 the value of nitrites as preservatives for fresh fish was first investigated (1), (2), in Canada and later their bacteriostatic action was studied. Subsequently the nitrites were used extensively in the Canadian Maritime Provinces for fillet preservation. Their use was legalized in Canada about 1949 (Canadian Food and Drugs Act). Nitrites have been used to a very limited extent in British Columbia, probably largely because fresh fillet production is relatively small and it has been pointed out that they exert a pro-oxidant effect in frozen fatty fish, but strangely enough an antioxidant effect in unfrozen fish (3). It is interesting to observe that nitrites have in recent years been used extensively in Norway to preserve herring prior to reduction (4).

It was long realized that nitrites, while undoubtedly very useful, were by no means the ideal fresh fish preservative. For this reason for many years an alternative was sought. Substances studied included penicillic acid, certain sulfa drugs, penicillin and streptomycin (5), (6). Early in 1950 it was reported that, of a number of substances studied including several antibiotics, aureomycin (chlortetracycline or CTC) was extremely effective in retarding bacterial spoilage of fish (7). It was soon found that this antibiotic was much more effective than any of 14 studied, and that it was very effective in about 1 part per million (p.p.m.) concentration in preserving both fish and beef (8). Higher concentrations were found necessary to preserve cooked crab meat (pH 7.9).

The discovery that the antibiotic CTC was an extremely effective preservative for flesh foods was followed by several years of rather limited development work. Deatherage, working independently with beef, reached the same conclusion regarding CTC and has been responsible for nearly all the work carried out on „red meats" (9), (10), (11). Much exploratory work on shrimp has been been carried out in the Florida, U.S.A. area (12). The Lederle Laboratories have been responsible for the application to poultry (13), (14), and Canadian findings for fish have been verified and extended for fish in California (15). A useful review covering much of this work has been published recently (16). One of the greatest difficulties met with in this work has been the defeatist attitude adopted by many, without any real consideration of the circumstances, that a „medical" antibiotic must never be used on foods destined for human consumption (*vide infra*).

CTC has been studied almost entirely because it has proven considerably more effective than terramycin (oxytetracycline) or tetracycline. It has been found that the antibiotic can be readily incorporated in ice used for icing fish, and this method would appear the most desirable since fish spoilage bacteria would be affected very soon after fish are captured and iced. In initial tests flaked ice with 1.2 and 4 p.p.m. of CTC was studied and, with well iced fish, the 1 p.p.m. level proved reasonably

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effective (17), (18), though it is quite probable that the high level might be desirable in cases where fish are rather poorly iced. Experiments were carried out on salmon trolling boats off the West coast of Vancouver Island, and fish which were subsequently transported to Vancouver were examined over a period of several months during the summer of 1954. Flaked ice containing 1 p.p.m. of CTC proved greatly superior to ordinary ice in these experiments, and a convenient method of adding CTC solutions to machines used for making flaked ice was described briefly (19), (20). Eviscerated fish dipped in 15 or 50 p.p.m. solutions of CTC and then iced with ordinary ice keep much better than fish iced directly with ordinary ice.

Though CTC may be added to flaked ice quite readily, much of the ice made is of the block type, and there is every evidence that this prodedure may continue for a long time at least, because of the convenience and necessity of storing large quantities of ice and the cost of reconverting large cold storage plants. Methods of distributing CTC uniformly through block ice so that it would not migrate to a center „core" were devised and have proven eminently satisfactory in practice. Several hundred tons of ice containing 1 p.p.m. of CTC have been made using these methods. The techniques are very simple and involve the use of substances such as carrageen and carboxymethylcellulose (about 0.005 of 0.01 %) as „distributing" agents. Various methods have been described (21), (22), (23), and a Canadian Public Service Patent for the general method is being applied for. Lederle Laboratories have done independent work in this field which has proved valuable in developing „Acronize B.I." (Acronize block ice) for use in ice plants (24).

The problem of assay for small amounts of CTC in fish flesh has proved more complicated than was at first anticipated. For ice a spectrophotometric assay for CTC is convenient and rapid (24), though in this work microbiological assays have been mainly used. Fish flesh presents a very different problem, for most species which were examined give a „fictitious" assay in the usual 'pad plate' microbiological assay procedure (18), (20). Difficulties encountered in recovery of trace amounts of CTC added to fish flesh have been reported recently (25). Fortunately flesh of one of the common food fishes found off the British Columbia coast (*Gadus macrocephalus*) has been found to give the fictitious assay only very occasionally, therefore flesh of this fish was found extremely useful in all the experiments which have dealt with penetration of CTC into fish stored in ice or sea water containing the antibiotic, and with its destruction by heat as in cooking. Certain of the results in these studies have already been reported (25), others have been submitted for publication (26) or are as yet in progess. These studies so far indicate only slight penetration of CTC from ice, and this largely in the skin or „belly flaps" (flesh surrounding the visceral cavity). The residual antibiotic activity is largely or entirely removed by heating as in cooking. In connection with this work the assay for CTC has been somewhat modified with a considerable increase in the sensitivity.

Although the initial research on use of CTC in flesh foods has been carried out in Canada, the first permission to use this in a food destined for human consumption was in the U.S.A. (27). After detailed submission of authentic scientific reports it was finally concluded that 7 p.p.m. of CTC could remain in flesh of uncooked poultry

without any danger to the consumer since this concentration, and indeed considerably higher concentrations, would be removed entirely by any normal cooking prodedure.

Public health aspects of CTC treatment of flesh foods

There is absolutely no doubt that use of the low concentrations of CTC indicated in the present report would never present the slightest danger to human health. In this respect sodium nitrite presents considerably more danger. Ingestion of, say, 1 gram of sodium nitrite, would be fatal, while the normal adult readily tolerates at least 1 gram of CTC daily, usually without any ill effect. A review on this subject has recently been prepared (28) [1]. Suffice it to say that prolonged oral administration of relatively small amounts of CTC, and in some instances of even massive doses, has not been accompanied by serious lasting toxic reactions. In a recent article the harmlessness of properly controlled use of CTC as a food preservative has been admitted by the American Medical Association (29).

This present work is not only concerned with practical studies in use of CTC in fish preservation, but also with its mode of action. It is hoped that CTC will replace nitrite as a legal fish preservative in Canada. To prevent its use would seem ridiculous since, in order to receive an average small daily normal oral dose of CTC from *uncooked* fish which had been iced with CTC-containing ice, several tons of the fish would have to be consumed. If the fish were cooked by customary procedures the residues of CTC would either be so small as to require consumption of over 5 tons of fish for a single oral dose, or there would be no CTC present. On cooking fish materials CTC is changed into microbiologically inert isomeric isochlortetracycline.

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RÉSUMÉ

L'UTILISATION D'AGENTS DE PRESERVATION ET DES ANTIBIOTIQUES POUR CONSERVER LE POISSON FRAIS

L'auréomycine (chlorotétracycline ou CTC) est extrêmement efficace pour retarder la putréfaction bactérienne du poisson. Elle est beaucoup plus efficace que la terramycine (oxytétracycline ou OTC) ou la tétracycline. Des expériences ont montré que la CTC est efficace à des concentrations de 1 p.p.m. quand elle est incorporée la glace. Dans des expériences avec des saumons pêchés au large de l'île Vancouver (Canada), on a trouvé que la glace en écailles contenant 1 p.p.m. de CTC est supérieure à la glace ordinaire. On a mis au point une méthode pratique pour l'addition de solutions de CTC aux machines servant à

la fabrication de la glace en écailles. Des poissons éviscérés, trempés dans des solutions de 25 à 50 p.p.m. de CTC et mis ensuite dans de la glace ordinaire, se conservent beaucoup mieux que des poissons mis directement dans de la glace ordinaire. La glace on blocs est d'un entreposage plus facile, et on a établi des méthodes pour distribuer uniformément la CTC dans tout le bloc. Plusieurs centaines de tonnes de glace contenant de la CTC ont été fabriquées. L'addition de 0.005 à 0.01 % de mousse d'Irlande et de carboxyméthyle cellulose comme agents distributeurs assure la dispension uniforme de l'antibiotique. Les laboratoires Lederle ont mis au point l'„Acronize B.I." pour l'utilisation dans les fabriques de glace.

L'essai spectrophotométrique de l'auréomycine dans la glace est une méthode rapide et pratique, et on peut aussi utiliser la méthode microbiologique d'essai. Cependant, avec le poisson on obtient parfois des valeurs fictives. Des recherches sur la pénétration de la CTC dans la chair de poisson ont montré que seules de petites quantités pénètrent dans la peau ou les parois abdominales. L'antibiotique résiduel est enlevé pendant la cuisson. Aux E.U. on autorise officiellement jusqu'à 7 p.p.m. de CTC dans la volaille crue.

La présence de faibles concentrations de CTC dans le poisson ne présente aucun danger pour le public. Même de fortes doses de CTC ont été tolérées sans aucune réaction toxique sérieuse, et pour ingérer une dose quotidienne orale normale de CTC il faudrait consommer plusieurs tonnes de poisson cru. La cuisson des produits de la pêche transforme la CTC en isomère isochlorotétracycline microbiologiquement inerte.

EXTRACTO

USO DE PRESERVATIVOS Y DE ANTIBIOTICOS EN LA PRESERVACION DEL PESCADO FRESCO

La aureomicina (clorotetraciclina o CTC) ejerce un efecto aun más poderoso que la terramicina (oxitetraciclina u OTC) o la tetraciclina para retardar la alteración bacteriana del pescado, demonstrando una gran eficacia durante los experimentos cuando se añadió al hielo en proporción de 1 p.p.m. Como en las experiencias con salmón pescado frente a la isla de Vancouver, C.B., Canadá, dicho refrigerante en forma de escamas dió mejor resultado que el de tipo común, se ideó un método adecuado para agregar disoluciones de CTC a las máquinas que lo fabrican. El pescado sin vísceras, sumergido en disoluciones de 20 á 25 % de CTC y colorado inmediatamente en hielo corriente, se conserva mejor que sise conserva solamente con este refrigerante, sin recurrir al tratamiento previo antes mencionado. Como el hielo corriente es más adecuado desde el punto de vista del almacenamiento, se idearon varios procedimientos para distribuir uniformemento el CTC en la barra; con este objeto se han fabricado varias tons, de dicho refrigerante en el cual se incluyó el antibiótico mencionado. La adición de agentes dispersantes como la carragenina y la carboximetilcelulosa a razón de 0,005 a 0,01 % aseguran la distribución uniforme de estas substancias. Los „Lederle Laboratories" han inventado el producto conocido con el nombre de „Acrónize B.I." para usarlo en las fábricas de hielo. El análisis espectrofotográfico de la aureomicina en este refrigerante es un procedimiento rápido pero también puede usarse la determinación microbiológica; sin embargo, en el caso del pescado a veces se obtienen valores ficticios. Los estudios sobre la penetración del CTC en la carne de estos animales han demostrado que sólo pequeñas cantidadas pasan a través de la piel de los costados o paredes abdominales y el residuo de antibiótico puede eliminarse durante la cocción. En E.U.A. se permito oficialmente hasta 7 p.p.m. en las aves crudas. La presencia de bajas concentraciones de CTC en el pescado no ofrece peligro desde el punto de vista sanitario, ya que el organismo puede tolerar grandes dosis de este producto sin serias reacciones tóxicas, y para ingerir una dosis normal de CTC por vía oral sería preciso consumir diariamente varias tons de pescado crudo. La cocción de la carne convierte a este compuesto en un isómero microbiológicamente inerte, llamado isoclorotetraciclina.

FRESH FISH PRESERVATION

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There is no doubt that more edible marine products are discarded as unfit for human food purely as a direct result of bacterial spoilage than from any other known factor or combination of factors. There has been a vast number of scientific papers published during the past few decades which have described types of bacteria associated with spoiling fish. Though it would be wrong to state that these are valueless, it is certain that they are of rather limited value because the systems of bacterial classification are very unstable. There are probably many thousands, or hundreds of thousands, of bacteria associated with spoiling fish which differ from one another sometimes very radically and sometimes in only one or two very minor ways. Intelligent attempts to divide these bacteria into groups according to their chemical requirements and their metabolic processes (1) will give a better understanding of their relative importance than do purely morphological and cultural characteristics. It is to be hoped that these metabolic studies will eventually lead to more scientific methods of controlling bacterial spoilage than those presently available.

It was in 1929 that Dr. ERNEST HESS was working actively on the problem of the influence of temperatures in the region of 0°C (32°F) on the rate of bacterial spoilage of fish, using the knowledge that fish will spoil much less rapidly in sea water chilled with ice (about –1°C or 30.2°F) than when stored in crushed ice at 2° or 3°C (35.6° of 37.4°F). This work (2) (3) was indoubtedly one of the most significant and fundamental contributions to our knowledge of fish spoilage, for it was shown that fish would spoil about twice as fast at 2.5°C (36.5°F) as at –1.1°C (30°F). The reason for this was the fact that fish, unlike meats from warm-blooded animals, is naturally contaminated with psychophilic bacteria, some of which will grow at -7.5°C (18.5°F) (4). Unfortunately little use has been made of the above observation by most practical fish handlers. It is of interest that in 1920 LE DANOIS secured a French Patent (5) for holding fish in chilled sea water at –4 C (24.8°F). This temperature could not be reached without addition of more salt to ordinary sea water, and would, moreover, result in undesiable slow freezing of fish.

STORAGE AND TRANSPORT OF FISH IN REFRIGERATED SEA WATER

The lowest temperature at which fish may be stored without their muscle tissues freezing is about -1 C (30.2°F), and it is this temperature which must be attained and fairly carefully controlled if one is to store fresh fish ideally. Unfortunately it has not proven easy to find conditions under which this is practicable, though various methods have been proposed. These have included scattering salt over ice used for icing fish, making flaked ice with a melting point of about –1°C (30.2°F) from sea water or eutectic block ice from 2 or 3% solutions of sodium bicarbonate or disodium phosphate, and storing fish or fillets, which are well protected by moisture-proof wrapping materials, in circulating air at –1 C (30.2°F).

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In a report published in 1947 (6) it was pointed out that, 'Clean sea water (or a 3% salt brine) cooled with crushed ice or by other means will have a temperature of about 29°F (–1.7°C) and might be of value in fish wells similar to the refrigerated brine wells used in tuna boats'. A few years later experiments were carried out on salmon trolling boats in which the fish were stored in tanks containing circulating sea water which was mechanically refrigerated (7), (8), (9). These initial trials proved highly successful and several privately-owned salmon trolling boats operating along the British Columbia and Alaska coast lines have been equipped with refrigerated sea-water tanks. Last year the Industrial Development Service of the Canadian Department of Fisheries provided and installed equipment for storing 40,000 to 50,000 lb. (18,140 to 22,680 kg) of fish on a company owned fish-packing vessel (10). As is usual with experiments of this type the fishing industry were, in general, rather sceptical, and it was therefore quite gratifying when the installation proved successful. It is important to note that several of the large companies in the Seattle area are so enthusiastic about this method, particularly as it applies to transport of salmon for canning, that they have already operated several very large installations on scows, and are apparently constructing others. At present installation of six large mechanically refrigerated tanks on a newly built halibut vessel is being supervised. The cost of this equipment is being provided by the Department of Fisheries Industrial Development Service. The vessel will be able to carry about 100,000 lb. (45,360 kg) of fish. The value of refrigerated sea water shore storage tanks for fish will also be investigated, particulary as the procedure affects the quality of salmon which must be stored prior to canning.

During the past year studies have been initiated to determine the effect of storage in refrigerated sea water on the fish stored therein. Certain of the results have been made the subject of various preliminary reports (10), (11), (12), (13), (14). The various factors examined have been the influence on bacteriological keeping quality, salt penetration (changes in Na+ and K+ ions), enzymic proteolysis ('belly-burn'), color of salmon and formation of 'curd' during canning of salmon.

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RÉSUMÉ

LA CONSERVATION DES PRODUITS DE LA PECHE FRAIS

Il y a plus de produits marins comestibles rejetés comme inconsommables à cause de la putréfaction bactérienne qu'en raison de n'importe quel autre facteur connu. L'entreposage des produits de la pêche à des températures juste au-dessus du point de congélation, i.e. —1°C, augmente beaucup la durée de conservation. Le poisson s'altère environ deux fois plus vite à 2,5°C qu'à —1,1°C. Mais jusqu'à présent on a peu utilisé cette observation dans la pratique. La plus basse température à laquelle le poisson peut être entreposé sans congeler les tissus musculaires est de —1°C environ. Une méthode pratique pour entreposer le poisson à cette température est d'utiliser de l'eau de mer ou de la saumure à 3 % réfrigérées. Des expériences à bord de bateaux pêchant le saumon à la traîne, sur lesquels on a installé des réservoirs d'eau de mer ayant une capacité de 40.000 à 50.000 lb. (18.140 à 22.680 kg) de poisson, ont donné d'excellents résultats. L'entreposage du poisson dans de l'eau de mer réfrigérée a aussi été utilisé pour le transport et l'entreposage du saumon pour la mise en conserve. Un bateau récement construit, destiné à la pêche du flétan, est muni de six grands réservoirs à eau de mer réfrigérés mécaniquement, ayant une capacité totale de 100.000 lb. (45.360 kg) de poisson.

EXTRACTO

PRESERVACION DEL PESCADO FRESCO

Como resultado de la deterioración bacteriana se eliminan más productos marinos comestibles que por cualquier otro factor. El almacenamiento del pescado a temperaturas inmediatemente superiores al punto de congelación, i.e., —1°C (30.2°F) prolonga considerablemente el mantenimiento de la calidad. El pescado se echa a perder en la mitad de tiempo a 2,5°C (36,5°F) que a —1,1°C (30°F); sin embargo, hasta ahora, en la práctica se ha hecho poco caso de esta observación. La temperatura mínima para almacenar el pescado sin que se congele fluctúa alrededor de —1°C. Un método práctico que permite almacenar el producto a este nivel calórico consiste en usar agua de mar o salmuera al 3 %. Los experimentos a bordo de los barcos de pesca con curricán, en los cuales se instalaron estanques con capacidad para 40.000 a 50.000 libras (18.140 a 22.680 kg) de pescado han demostrado ser muy satisfactorios. El almacenamiento en agua de mar refrigerada también se ha usado para el transporte y almacenamiento del salmón destinado a las fábricas de conservas. A bordo de un nuevo barco recién construído para la pesca de hipogloso también se han instalado 6 tanques para agua salada con una capacidad total de 100.000 lbs. (45.360 kg) de pescado, donde el agua de mar se refrigera por medios mecánicos.

New Horizons in Food Preservation

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Reprinted from

CANADIAN FOOD INDUSTRIES

Part I, May 1959. Part II, June 1959.

FRB No. 67

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►*Man has preserved foods for use in times of adversity or scarcity for centuries by the simple and obvious methods of drying, salting and smoking or by combinations of these. Food is still preserved by these methods in localities where newer procedures are not available. Some of the more modern methods of preserving foods, their methodology, present applications and, so far as I can foresee, their future potentials will be outlined in the ensuing text.*

Canning

The canning of foods is one of the most valuable discoveries made by man during the past 150 years. In 1809 Nicholas Appert received a prize from the French Government for his discovery of the art of canning. His method was simple for he sealed meat or fish, fruit, eggs, etc. in air-tight bottles, wiring on the stoppers, and immersed them in boiling water for various periods. About 1860 Louis Pasteur made certain discoveries which stimulated the general procedure of food preservation by canning, but it was some time before his contributions were recognized.

Between 1850 and 1900 various patents were granted for processes involving sterilization of foods by steam under pressure, and since this time canning has become one of the most important methods of preparing foods, and many millions of dollars have been spent on research to improve canning methods and to make products more palatable and entirely safe. The heating conditions necessary to destroy anaerobic spore-formers, such as *Clostridium botulinum*, and, in vegetable packs, thermophilic organisms causing flat sour fermentations have been established.

It has been claimed that botulinus poisoning from commercially canned fruits has not occurred on this continent since 1925, though of course there have been numerous outbreaks occasioned by consumption of improperly home-processed foods. *Clostridium botulinum* will rarely develop in those canned foods, the reaction of which is below pH 4.5. Fruits usually have lower pH values than this and do not require very rigorous heat treatment. On the other hand, the reaction of canned vegetable packs is above pH 4.5 and much more rigorous heating procedures are required.

Prolonged heating at high canning temperatures almost invariably cause greater losses of colour, flavour, texture and vitamin and other nutritive value properties of canned foods than do relatively high temperatures short-time (HTST) methods. Consequently, considerable research has been directed toward the perfection of HTST methods of canning. It is easier to develop HTST processes for liquid or semi-solid foods than it is for solid foods, and more difficult to evolve such processes for foods which might readily be damaged by movement in the can. In general, the destruction of bacteria increases ten-fold for each 10°C. rise in processing temperature, whereas chemical reactions associated with organoleptic deterioration are generally only doubled for each such 10° rise in temperature. The following is a very brief review of some HTST processes.

(1) *High temperature short-time processing followed by aseptic filling and closing of the containers.* This category includes the so-called HCF process, the Avoset process, which was used for canned cream about 1942, the Martin process, which is rather similar to the HCF process, and the Smith-Ball process. Pre-sterilized tomato juice has become an accepted product and about 100 different installations for its preparation are used today.

(2) *Agitating processes for liquid and semi-liquid products in the can.* The purpose here is to increase the rate of penetration of heat into the can by agitation. The Green Giant Company have used a thermo-roto machine in which the cans are sprayed with a high boiling liquid such as ethylene glycol. Due to the extreme pressures generated in the cans, a heavy plate is required for their construction. Machines which agitate or roll cans during retorting are the Anderson-Barngrover cooker and those used in the Agitort process which includes an end-over-end rotation of the cans.

A system used to some extent in Great Britain employs a continuous type of retort in which a "barometric leg" is used to attain the required temperature. These new processes perhaps would be more widely used if equipment costs were not so high. There is a great need for further research in canning and the potentialities of this method of food treatment are by no means exhausted. In certain countries aluminum cans are used, and continual research is being done concerning new types of enamels to prevent can corrosion for marine products, etc.

Refrigeration and Freezing

During the past 40 years there has been a spectacular increase in the amount and variety of foods treated by chilling and refrigeration procedures. Thus, we in Canada may obtain fresh fruits and vegetables from the south.

Foods to be treated by chilling or freezing methods should be processed as soon as possible after harvesting and consequently considerable attention has been given to various pre-cooling techniques. Vacuum cooling of lettuce before shipment is now used. Hydro cooling by chilled circulating or sprayed water of citrus fruits, peaches, etc., for export is employed extensively. Mechanically refrigerated railway cars are used for many products, particularly frozen citrus fruits concentrates, but not enough of these cars are available to meet all demands.

When loading refrigerated trucks or cars, products should be at about the same or at lower temperatures

than those used for shipment, for these vehicles are not designed to freeze or chill products efficiently. Meat products are usually promptly chilled or frozen at the slaughterhouse. Fish should be iced promptly after capture and evisceration. Due largely to the initiative of the Vancouver Technological Station of the Fisheries Research Board of Canada, the method of storing and transporting fish in refrigerated sea water at 30°F. (a temperature which is very slightly above the freezing point of fish flesh) is becoming increasingly popular. This method is very valuable, offers many advantages and has few disadvantages.

Chilled foods are perishable and usually have a limited "shelf life". It was once thought that frozen foods could keep almost indefinitely without deterioration so long as they remained "frozen", but the fallacy of this opinion has long been recognized. Flesh foods, particularly fish, are quite perishable even when stored in a frozen condition. One of the most serious problems in the storage of frozen flesh foods is the development of oxidative rancidity. Possibly this change is accelerated if the fat has been split by lipolytic enzymes to yield free fatty acids. Oxidative rancidity may occur in practically all flesh foods and more particularly in those containing long-chain polyunsaturated fatty acids. Fish, shellfish and whale meat are among the foodstuffs rich in such acids.

So far as is known, the only catalytic effect present in these flesh foods is that due to so-called haemin catalysis of fat oxidation, and no true lipoxidase type of enzyme appears to be present. There are only two known techniques available for inhibiting oxidative rancidity in frozen foods, namely, (1) *exclusion of atmospheric oxygen*, and (2) *storage at very low temperatures*. Though it has been known for about thirty years that frozen fish should be stored at about —28°F., few, if any, storages maintain temperatures much below 0°F. Exclusion of oxygen from frozen foods is accomplished by glazing whole fish or steaks of fish, and by packaging meats and fish products in wrapping materials that are relatively impermeable to oxygen, in some cases with the aid of vacuum and nitrogen. These procedures improve quality, colour, etc. of frozen flesh foods.

Many of the deteriorative changes which occur in frozen fruits and vegetables have not yet been explained but are also usually controlled by storage at low temperatures. Some varieties deteriorate less in storage than do others. One of the most common errors in handling frozen foods is improper use of display cabinets. These may be so overloaded that there is not proper air circulation. Foods are not infrequently allowed to defrost and slowly refreeze in such cabinets with resultant serious loss of quality.

A common defect of frozen flesh foods, and more particularly of fish, arises from the denaturation of an important muscle protein, myosin, which in turn results in dryness and toughening that is most unpleasant to the consumer. This denaturation is not fully understood and the only known control method is fairly rapid freezing and storage at low temperatures, such as —28°F. At 0°F. fish products gradually become tough within a few months. It is hoped that continued research will eventually provide a satisfactory solution to this problem.

Dehydrofreezing and Dehydration

In essence, dehydrofreezing is conventional freezing with dehydration interposed in the processing sequence just before packaging and freezing for storage. So far, this process has been applied only to fruits and vegetables and that on a minor scale and chiefly for institutional packs. Thus, in 1955 in the United States, about 210 million pounds of pears, carrots, apples and apricots were prepared by this method, largely for institutional packs.

Dehydrofreezing involves an initial partial dehydration to about 50 per cent of the original material weight followed by conventional freezing. One of the chief advantages is the decrease in bulk as compared with frozen products, while the products are more palatable than conventionally dehydrated products. Flavour, texture, appearance and colour are improved, and lower sulphite levels are required than for ordinary dehydrated fruits.

Dehydrofrozen peas are considered as palatable as frozen peas, when both are prepared from the same raw material. With some fruits, especially apricots and freestone peaches, troublesome changes in cell-wall permeability retard optimum dehydration, but this does not apply to peas, apples and carrots. Dehydrofreezing is evidently impractical for commodities that cannot be rapidly dehydrated to at least one-half their original weight without adverse changes (e.g., lima beans and corn).

One of the difficulties involved in dehydrofreezing is that of obtaining uniform drying with commodities of non-uniform size. Since the air velocity may attain 400 cu. ft./min./sq. ft. of bed area, and the depth of diced vegetables, etc. may be 2.5 to 3.5″, air temperatures of between 200 and 300 F. may be used without damaging the product. To date the most suitable equipment for this purpose is an experimental belt trough type of dryer. The cost of preparation, packaging, storage and transportation for dehydrofrozen fruits and vegetables is lower than that for similar frozen or canned products. Thus, for institutional markets and remanufacturing uses, dehydrofrozen products have useful possibilities.

With the exception of certain products such as dehydrated eggs, milk and some fruits, dehydration has failed to yield really acceptable food products other than those which might have value in emergencies. Furthermore, it is only fairly recently that dehydrated milk and egg products have shown marked improvement. This is largely because research during World War II showed that much of the deterioration was caused by so-called non-enzymic 'browning' or protein-sugar (Maillard) reactions, and that these could be controlled either by reducing the moisture content to an extremely low level, or in egg products by removal of glucose by glucose oxidase.

Dehydration of flesh foods by cooking followed by air-flow drying has usually yielded more or less unpalatable products. Consequently much effort has been directed toward methods of obtaining satisfactory freeze-dried fish or meat products. However, freeze drying is probably usually a fairly expensive method of food preparation. One serious problem is the formation of a layer of dried fish on the surface which acts as an insulator and water-vapour barrier to the remainder of the fish or meat product. Infrared heating of the product during freeze drying has not proved satisfactory. Microwave heating to accelerate the transfer of water vapour from the flesh material to the refrigerated surface is being investigated. Canadian experiments have included use of metal prongs which penetrate the flesh to act as heat conductors. It would seem highly doubtful whether there is a compelling reason to prepare freeze-dried flesh foods except for possible emergency use.

Chemical Preservation

Under this heading will be considered not only chemical preservatives but also antioxidants and antibiotics.

These must be considered under this category for they are essentially chemical compounds. Some of our modern food-processing methods have rendered obsolete the use of chemical preservatives, but there are still instances where their use is almost mandatory under present conditions of preparation, distribution and storage.

In general, Pure Food Regulations in Canada and the United States permit only relatively few food additives and these may be added only to certain foods under specified conditions and at specified concentrations. Examples are benzoic acid, sulphurous acid, sorbic acid, propionic acid and their salts, gum guaiacum, lecithin, citric acid, tartaric acid, ascorbic acid, propyl gallate, butylated hydroxyanisole and nordihydroguiaretic acid, chlortetracycline and oxytetracycline. Some of the interesting uses of certain of these substances will be considered.

Preparing Antibiotic Ice.

Sulphurous Acid and Ascorbic Acid

The solution of the problem of enzymic browning in the frozen fruit industry has been aided by the use of ascorbic acid. The browning of fresh and frozen fruits is due to the action of the enzyme polyphenolase which combines with molecular oxygen and suitable phenolic substrates to yield brownish reaction products. The substrates involved in enzymic browning include hydroxyphenols and related substances. Thus, catechol tannins, dihydroxycinnamic acid, caffeic acid, protocatechuic acid, etc. have been known to be involved. Certain reducing agents, particularly ascorbic acid, act as preferential oxygen acceptors and thus prevent the enzymic browning reaction.

In the processing of dehydrated fruits ascorbic acid is of little value because it is so readily oxidized that it does not remain long enough in contact with the tissues to exert any lasting beneficial effect. Sulphur dioxide is the most useful agent for the prevention of browning reactions in dried fruits that has been discovered as yet. It is probable that prevention of browning in dried fruits by sulphite treatment involves both Maillard, or non-enzymic browning reactions, as well as the enzymic reactions.

In the dehydrated fruit industry the sulphur dioxide levels used may range from about 800 to 2000 p.p.m. (.08 to 0.2 per cent). The inhibition of non-enzymic browning in dehydrated fruits by sulphur dioxide is largely due to binding of the reactive aldehyde groups of reducing sugars so that they are unable to react with amino groups of nitrogenous compounds. Much work remains to be done on this problem since little is known concerning these nitrogenous compounds.

One of the most important uses of sulphur dioxide is in the wine industry. It has been used in Europe in the manufacturing of wines at least since 1905. The main reason for using sulphur dioxide in wine making is because it prevents growth of undesirable lactic acid and acetic acid or vinegar bacteria, and it also suppresses undesirable wild yeasts (torulae) which may spoil the flavour of the wine. Typical good wine yeasts soon become adjusted to sulphur dioxide, and may be trained to withstand quite high concentrations.

Higher concentrations are required for unsound, bruised and older grapes than are required for fresh clean ripe grapes. The concentrations which are used vary from about 75 to 200 p.p.m. Much of the added sulphur dioxide is bound by the aldehyde groups of reducing sugars such as glucose and by other aldehydes, such as acetaldehyde, which may be formed during fermentation. Some is lost during fermentation with the carbon dioxide that is evolved. Research may result in the discovery of some inhibitor preferable to sulphur dioxide. A few processors maintain that sulphur dioxide causes some impairment of the natural wine flavour.

Antioxidants

Research on antioxidants and their applications has increased considerably during the past 20 years. Substances which are most commonly permitted for use in edible fats and shortenings include propyl gallate, d-isoascorbic acid and l-ascorbic acid and certain of their esters, thiodipropionic acid, gum guaiac, nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole and butylated hydroxytoluene. Stabilization of fish oils and of cured or frozen flesh foods with certain antioxidants has also been investigated. Butylated hydroxyanisole and butylated hydroxytoluene have proved particularly effective with fats which are used in preperation of baked goods, since they are sufficiently heat stable to possess a "carry-over".

Propyl gallate and closely related compounds, while excellent antioxidants, are not sufficiently heat stable and contact with iron must be carefully avoided to prevent formation of purple colour complexes. Naturally-occurring compounds, such as a-tocopherol (vitamin E) and lecithin, act as antioxidants and are often valuable since they may form so-called synergistic combinations with other added antioxidants. Since oxidation of fats is often accelerated by heavy metals such as iron, copper, etc., chelating agents, such as citric and phosphoric acids, have proven extremely valuable as additives in synergistic or other combinations of antioxidants.

Preservation of Food by Antibiotics

There is no clear division between antibiotics and other chemical substances used in food preservation. As an extreme example the inorganic chemical sodium nitrite, which has been used extensively for fish preservation in eastern Canada, is also a product of microbial metabolism and therefore could rightly be termed an antibiotic. On the other hand, some antibiotics have been chemically synthesized.

The first reported attempts to use an antibiotic for food preservation were made about 1943, when penicillic acid, a mould metabolism product, was investigated for the preservation of fish. A few years later penicillin and streptomycin were investigated for the same purpose. None of these antibiotics proved satisfactory. Other early attempts included use of penicillin to prevent milk decomposition by spore-forming organisms. Since then, research concerning preservation of a variety of foods with antibiotics has increased considerably. This has continued in spite of the grim prognostications and dire warnings of the overly-cautious individuals that there could be no future for applications in this area. Probably these same individuals would have opposed the practical application of the results of early experiments which eventually led to the use of antibiotics to stimulate growth of young farm animals.

Food preservation by use of antibiotics is no simple subject, for not only are a very wide variety of foods involved, but the micro-organisms which must be controlled vary with the foods in question. For examples, problems could include control of bacteria in alcoholic fermentation, of yeasts and moulds in meat and in smoked fish, of organisms which cause rot in chilled packaged fruits and vegetables, and of psychrophilic, mesophilic and thermophilic bacteria which cause fish and meat to spoil. Each type of spoilage represents a specific problem and requires its own careful analysis and solution. The largest volume of research and the largest application of antibiotics to food preservation has been made with flesh foods, and consequently I shall discuss this work in some detail.

In 1950 it was found that the wide spectrum antibiotics, namely chlortetracycline (Aureomycin), exytetracycline (Terramycin) and chloromycetin were, in the order named, the most effective antibiotics for flesh food preservation. Subsequent research has confirmed and extended these original findings.

The first work with chlortetracycline was carried out with fish but it was not until November 1955 that the United States Government permitted use of not over 7 p.p.m. in uncooked poultry. The results of research concerning preservation eviscerated poulty with chlortetracycline as carried out by U.S. investigators have recently been verified in Britain. However, British investigators have found that this treatment is ineffective with British poulty, which is rarely eviscerated. They have fed high levels of tetracycline antibiotics to poultry just prior to slaughter, and this certainly controls some of the visceral spoilage which occurs in non-eviscerated poultry such as is sold in Great Britain. To date, hundreds of thousands, and probably millions, of pounds of chilled eviscerated poultry have been treated with chlortetracycline in the United States with highly satisfactory results. Where occasional unsatisfactory results have been reported, unsanitary plant procedures or improper applications have been to blame. Thus, chlortetracycline has been used in a poultry plant that was purposely operated under unsanitary conditions, and within a short time the treatment soon became useless because of the generation of resistant micro-organisms. This observation shows that it is absolutely essential that an antibiotic treatment of flesh foods should be carried out only under sanitary conditions and with fresh flesh foods, and that all equipment should be thoroughly cleaned or sterilized, so that no resistant organisms may develop.

In Canada the use of 5 p.p.m. of chlortetracycline for raw fish was permitted under the Food and Drug Regulations in early October of 1956, and since that time the use of chlortetracycline and of oxytetracycline has been permitted for both fish and poultry. Since the first Canadian investigations further studies on chlortetracycline preservation have been carried out in other countries, mainly in the United States, Japan and Great Britain and, to a lesser extent, in the Scandinavian countries, Russia, South Africa, etc. The results all indicate that 1 to 5 p.p.m. of chlortetracycline incorporated in either flake or block ice used for preserving fish extends keeping quality, as compared with that of fish iced in the ordinary manner, by about 7 days.

The addition of 1-5 p.p.m. of chlortetracycline to refrigerated sea water or brines in which fish are transported or stored also improves keeping quality considerably. Dipping of fillets in 5 or 10 p.p.m. chlortetracycline solutions is also used to retard their spoilage. Chlortetracycline also suppresses certain organisms associated with food poisoning, notably *Clostridim botulinum* type E and enterotoxigenic staphylococci. The problem of antibiotic residues in treated foods has also been thoroughly investigated to meet requirements of Food and Drugs organizations. Cooking eliminates all measurable residues from poultry and all but traces, which are probably insignificant, from fish.

Research at Ohio State University has shown that preservation of red meats, usually by perfusion techniques, is quite practicable and brings about great improvement in their keeping quality. So far, the dangers which some had thought might be associated with application of tetracycline antibiotics to foods do not appear to have materialized. The chief danger in their use would appear to be applications carried out under unsanitary conditions.

Electro-magnetic Radiations for Preservation and Processing

The use of electro-magnetic radiations for food processing is not new since ultra-violet light has long been employed as a germicidal agent. Electro-magnetic radiations are composed of packets of energy known as quanta and travel in a straight line through space at a velocity of 3×10^{10} centimetres per second. The following is a brief discussion of the various frequencies which are of probable interest to the food processor.

Induction heating and dielectric heating (10^7 to 10^8 cycles per second) may be used to cook foods. Induction heating occurs when the substance treated is an electrical conductor and is placed in a varying magnetic field. When the substance or substances are naturally insulators, dielectric heating will occur, friction being set up in the molecules of foods held between positive and negative condenser plates. Radar energy has also been used to process or cook foods, at least experimentally. In this instance much higher frequencies are used and these fall between 1,050 and 3,000 megacycles. Infrared radiations, which fall just above the visible spectrum, have very low penetrating ability and any temperature rise which occurs would be on the surface or due to conduction from the surface of a solid. Ultraviolet radiations which occur just below the visible range cover a frequency band of about 10^{16} to 10^{17} cycles per second or from about 150 to 350 millimicrons.

In spite of much publicity they

have so far found rather limited use in the food field. They possess certain objectionable characteristics, such as the very painful effect they have on the operator's eyes, and they also tend to produce ozone which can be detrimental to fats if ultraviolet lamps are used in storage rooms. They have negligible penetrating power in most solids. However, they have a very pronounced germicidal power.

During the past decade many hundreds of millions of dollars have been expended on research to preserve foods by ionizing radiations. Ionizing radiations can energise individual atoms or molecules of a medium such as a food to such an extent that an orbital electron is ejected from the molecule and a positively charged ion remains. The use of electro-magnetic X-rays and gamma rays which have wavelengths between 10 and 10^{-4} Angstrom units and of the particulate radiations, namely cathode rays and beta rays, is considered technically feasible for food preservation. Those particulate radiations which have the property of bringing about nuclear transformations, such as the heavy particle radiations, alpha rays, neutrons, protons, deutrons and fission fragments, could occasion formation of radioactive atoms. Gamma rays are electro-magnetic radiations emitted by naturally occurring and artificial radioactive elements and to some extent resemble hard X-Rays of short wavelength. Beta radiation is also obtained from practically all radioactive elements and is important for sterilization or partial sterilization of foods.

It is impossible to go into any detailed discussion of the various forms of radiation and their advantages and disadvantages. This has been adequately covered in several good recent publications. Wastes from nuclear reactors offer a possible source of power for radioactive sterilization of foods. Fuel rods, during their cooling periods, and more especially fission product wastes, are also useful. Indeed, the latter represent a long-term embarrassment to operators of atomic energy plants, and if the wastes are not used for providing useful radiation they must be stored in such a way that the radiation is harmless to man. Any leakage, particularly into natural drainage water, must be entirely prevented. This is probably one of the reasons why so much money has been expended on the possibility of using this source of penetrating radiations for food sterilization.

What are the possibilities for actual use of penetrating radiations with foods In general, two types of techniques may be used, namely, total sterilization and partial sterilization ("pasteurization"). A dose of about 2 million rep. (roentgen effective physical) is adequate to sterilize most foods, and lower doses, as, for example, between a quarter of a million and one million rep., can be used for partial sterilization or pasteurization.

Penetrating radiations when applied to feeds may cause a variety of chemical changes, one of the most important of which is that of dissociation of water into free hydroxyl radicals and hydrogen atoms, the former being oxidizing agents and the latter reducing agents. It is for reasons of this kind that much thought and experimentation has been directed toward irradiation of foods in the frozen state or in the absence of atmospheric oxygen, and also in the presence of free radical acceptors such as ascorbic acid. Unfortunately, these techniques involve extra expense and difficulties, and are not always practicable.

Unfortunately, some investigators fail to emphasize the facts that flavour degradations commonly occur in treated foods, and that physical changes are noticed in some, such as a softening of treated vegetables. Changes and degradations occur in many important food constituents such as amino acids, proteins, pectins, carbohydrates, fats, vitamins, etc. There seems little reason to attempt to sterilize, or partially sterilize, foods such as dried fruits and vegetables which are probably fairly stable if sulphited and dried in the usual manner.

Flesh foods have been one of the main targets for experimentation, but these, unfortunately, are particularly susceptible to undesirable changes. Though some believe that there may soon be industrial application to foods, many responsible individuals who have carried out a lot of experimental work are extremely cautious and doubtful if radiation sterilization will be applied to meats, fish or poultry for many years, except in very limited cases As an example of the effect of pasteurizing doses of radiation as applied to meats, fish or poultry for many years, except in very limited cases. As an example of the effect of pasteurizing doses of radiation as applied to fish, recent unpublished experiments have indicated that doses of between a quarter and a half a million rep. cause off-odours in practically all samples of fatty and non-fatty fish, but that on cooking the fish, only the fatty fish have off-flavours. One important factor, namely, the effect of such light pasteurization doses on subsequent storage of frozen flesh foods, has not yet apparently been investigated. The scientist who was, up to last year, head of the food radiation section of the Quartermaster Food & Container Institute in the United States stated that radiation processing of foods would probably be of five types, namely, (1) sprout inhibition of tubers; (2) prevention of insect infestation in stored grains, etc.; (3) irradiation of meat products, specifically pork to kill trichinae; (4) pasteurization of milk, and (5) sterilization of foods.

It is possible that the pre-cooking of certain foods followed by irradiation may prove useful in that the cooking inactivates enzymes which certainly do, in some cases, cause deterioration. Over-enthusiasm for these applications should be tempered by the knowledge that there are a number of factors which must be considered. Among these are the high cost of equipment, the need for really skilled operators, undesirable flavour or side reactions which may occur, and the cost of transporting foods to central treatment depots where this is indicated as in sprout inhibition or grain infestation treatments.

In conclusion, it is apparent that, though much has been accomplished in the field of food preservation, there are yet many unsolved problems. It has been stated that research is the keystone to this technological age in which we live and that no progress can be made without it. Research is not a luxury, it is a basic n e c e s s i t y, and I cannot over-emphasize the need for fundamental research. Too many of our industrial people favour applied research entirely, not realizing that in the long run they must rely on basic new discoveries for progress. ◄

LIGHT ATTENUATORS FOR USE IN PHYTOPLANKTON PHOTOSYNTHESIS STUDIES

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A study of the response of phytoplankton to variations of light intensity is becoming increasingly important in primary productivity research, both in lakes and in the ocean. In all such work we need to illuminate bottles of water containing algae to a light intensity which is a known fraction of that of an incident light source which may be daylight or some artificial form of illumination.

It is more difficult than might be supposed to contrive a convenient attenuator, the coefficient of which is easily reproducible, can be varied over a wide range and is constant with time. The attenuator should be robust, unaffected by water and truly neutral in its spectral behavior. Fogged photographic film or plates, even with protective coatings, have few of the above desirable characteristics.

If light is blocked off by wire gauze of fine mesh size the attenuation is truly neutral and the system can be made robust and immersed in water for reasonable periods of time with no deterioration. Furthermore, provided that the mesh size of the gauze is sufficiently small, the effect of more than one layer of gauze is additive, that is to say if the fractional transmission of one layer of gauze is T the attenuation of two layers is approximately T^2 *etc.*, up to at least four layers.

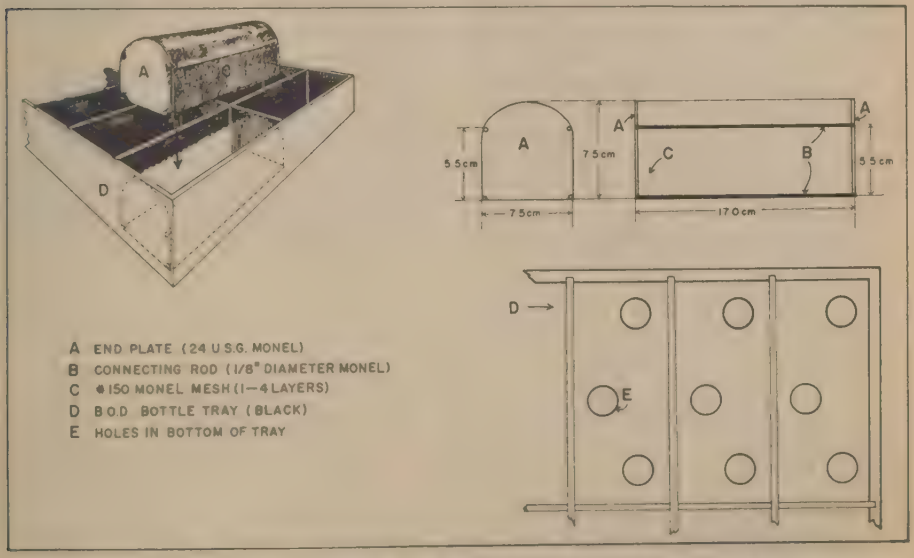

FIG. 1. A light attenuator, showing also its use in a tray from a water-cooled incubator.

Reprinted from LIMNOLOGY AND OCEANOGRAPHY, Vol. 6, No. 2, pp. 226–228, April, 1961.

FRB No. 6

This forms the basis of the neutral filters shown in Figure 1. The "light incubators" used by us for photosynthetic work are designed to hold black-painted trays with perforations in their base which allow cooling water to flow up through the equipment. The trays, which are illuminated from above, hold 300-ml B.O.D. (biological oxygen demand) bottles which we use for both oxygen and C^{14} photosynthetic rate measurements.

The filters consist of one or more layers of 150-mesh Monel metal screen wrapped over a Monel metal frame of rods and plates, as shown in the diagram, and soldered to the bottom rods and to the endplates. B.O.D. bottles fit snugly into this assembly which will rest in the compartments of incubator trays (as shown) or can be used anywhere where a flow of cooling water is available. Bubbles may tend to collect on the outer surface of the mesh when water is flowing but can be easily dislodged. As a precaution it is perhaps best to pass cooling water through a bag made of 150-mesh Monel screen before it enters the incubator but we have found no evidence of clogging or deterioration of filters in continual use throughout one summer. They should be rinsed with fresh water and stored dry.

The percentage transmission of light is surprisingly uniform over the base of a filter and amounts to about 35% for one thickness of screen, 13% for two thicknesses, 5% for three thicknesses and 2% for four thicknesses. The variation from filter to filter does not exceed 5–10% of the absolute amount and changes very little with time. We do not recommend the use of five layers of screen but a flat "filter" of 150-mesh Monel may be placed over one or more compartments in a tray. This effectively multiplies the transmission of each filter thus covered by a further 0.35.

The gap between 100% transmission and the first Monel filter (with 35% transmission) is rather large. However, a filter with about 65% transmission can be constructed using galvanized screen made from 16 × 12 mesh S.W.G. 33 wire. Doubtless similar materials could be found to give transmissions greater than 65%. Galvanized iron wire, protected by an acrylic spray treatment, has proved to have adequate corrosion resistance.

Attenuators should be calibrated *in situ* whenever possible. The exact fraction of the light cut out by the screens will vary somewhat with the equipment used and the nature of the light source, in particular whether or not the light reaching the screens is parallel or diffuse.

OCEANOGRAPHIC OBSERVATIONS IN THE CANADIAN ARCTIC AND THE ADJACENT ARCTIC OCEAN*

The history of oceanographic exploration in the Canadian Arctic does not coincide chronologically with that of geographic exploration. In the early stages of arctic sailing the urgency of the discovery of the Northwest Passage took full priority over any other interest and later, during the period of the Franklin Search, little time could be spent in scientific observations. Several of the early arctic captains recorded observations on the natural history of the country and on more than one occasion instruments and methods were developed to sample the bottom and measure the more obvious currents. All the early expeditions made frequent references to local ice and weather conditions and in areas where shoaling water was suspected the soundings were listed in the journals.

One of the first arctic expeditions for which there are records of oceanographic observations is that of Sir John Ross in 1818. On this cruise sub-surface temperatures were taken with a Six's thermometer at 80 and 250 fathoms in the eastern end of Lancaster Sound. For his oceanographic observations Ross designed a type of water bottle that closed at a predetermined depth and a device for bringing up a sample of the bottom sediments.

In 1819 Parry took sub-surface temperatures and recorded depths and ice conditions in Lancaster Sound and Barrow Strait and in 1845 the Franklin Expedition was equipped with a number of bottles and copper cylinders in which messages were to be sealed. These containers were to be dropped into the sea at specific locations north of 65° N.

The second Norwegian Arctic Expedition in the *Fram* was in the Canadian Arctic from 1898 to 1902. During this time plankton collections, surface temperatures and bottom samples were taken at various positions in the region of Jones Sound; however, no sub-surface temperatures were recorded since the ship was not equipped for this type of work.

The voyages of the C. G. S. *Arctic* from 1906 to 1911 under Captain Bernier contributed much significant information concerning the surface temperature, salinity, and bottom topography in Parry Channel as far west as Winter Harbour and in 1916 the Canadian Arctic Expedition made a number of tidal observations at Cape Kellett and at three locations on the north coast of Alaska. During the late twenties various spot oceanographic observations were made by observers carrying out investigations in marine biology for the Fisheries Research Board.

The Danish *Godthaab* Expedition and the work of the United States Coast Guard Cutter *Marion* inaugurated the recent phase of oceanographic research in the North American Arctic in 1928 [1,2]. These ships carried out extensive oceanographic observations in Baffin Bay, Davis Strait and the approaches to the channels leading into the Canadian Arctic Archipelago. The work was continued by the Hudson Bay Fisheries Expedition sponsored by the Department of Fisheries in 1930, and the United States Coast Guard Cutter *General Greene* carried out further investigations in Baffin Bay and Davis Strait between 1931 and 1935. Additional surface observations of temperature and density

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were taken by the Royal Canadian Mounted Police Schooner *St. Roch* during the period 1935 to 1937 in the channels forming the southern route of the Northwest Passage [3].

After the war arctic oceanographic investigations became more specific in character. The growing national interest in the North and the demand for information on the circulation, ice cover, ice movement, navigability, and the productivity of the waters led to the organization of a number of oceanographic cruises the result of which has been a vast accumulation of oceanographic observations within a very short time.

The Fisheries Research Board of Canada has been instrumental in conducting a large proportion of this work beginning with the cruise of H.M.C.S. *Magnificent* and *Haida* to Hudson Bay in 1949. The same year the fisheries research vessel *Calanus* began investigations in Ungava Bay, and in 1951 and 1952 the motor vessel *Cancolim* of the Defence Research Board carried out oceanographic observations in the western Arctic. The surveys conducted from the icebreaker *Labrador* since 1954 have covered a large area of the eastern Arctic and have resulted in the first sequence of oceanographic observations to extend from Baffin Bay in the east to the Pacific Ocean in the west [4].

Ships of the United States have also been active in collecting arctic oceanographic information. During the last 10 years seven American icebreakers have carried out oceanographic surveys in the channels of the Canadian Arctic Archipelago. Many of these surveys have been conducted in close co-operation with the Canadian investigations and all data

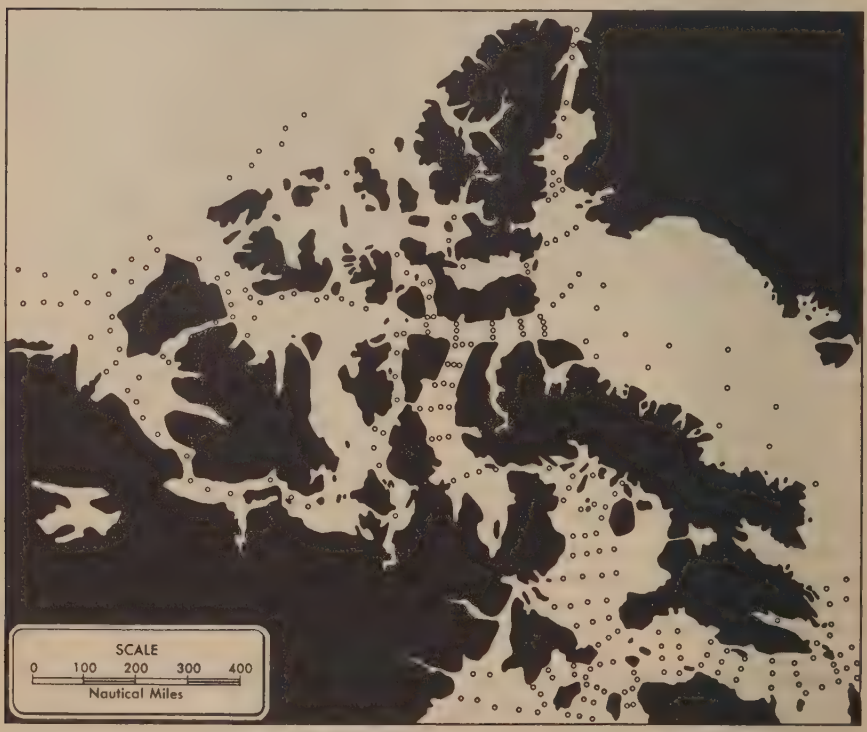

Fig. 1. Location of oceanographic stations.

have been freely exchanged between the two countries.

The most recent oceanographic programmes in the Canadian Arctic have been more confined in geographical coverage than the earlier reconnaissance surveys. Intensive investigations have been completed in Hudson Strait and Lancaster Sound by the Fisheries Research Board and the Hydrographic Service and in 1958 observations were taken from the I.G.Y. drift station T-3, which at that time was close to the western margin of the Canadian Arctic Archipelago.

Fig. 1 shows the distribution of oceanographic stations that have been taken in the North American Arctic within the last 30 years. The majority of the observations in the eastern region were taken from the Canadian icebreaker *Labrador* and the Fisheries research vessel *Calanus*. The stations in Kane Basin and Smith Sound were taken by the United States ships *Eastwind* and *Edisto*. In the western Arctic the United States ships *Burton Island* and *Requisite* and the Canadian motor vessel *Cancolim* have recorded most of the oceanographic observations.

A detailed bathymetric map of the region is unreliable since in many areas there is little or no information on the depth of water. Fig. 2, which is compiled from available sources, shows the general configuration of the bottom in the Canadian Arctic. Unfortunately, in the Queen Elizabeth Islands and the western region of Parry Channel the contours are based on only occasional soundings.

The deepest water in the Canadian Arctic Archipelago occurs in the eastern

Fig. 2. Bathymetric map.

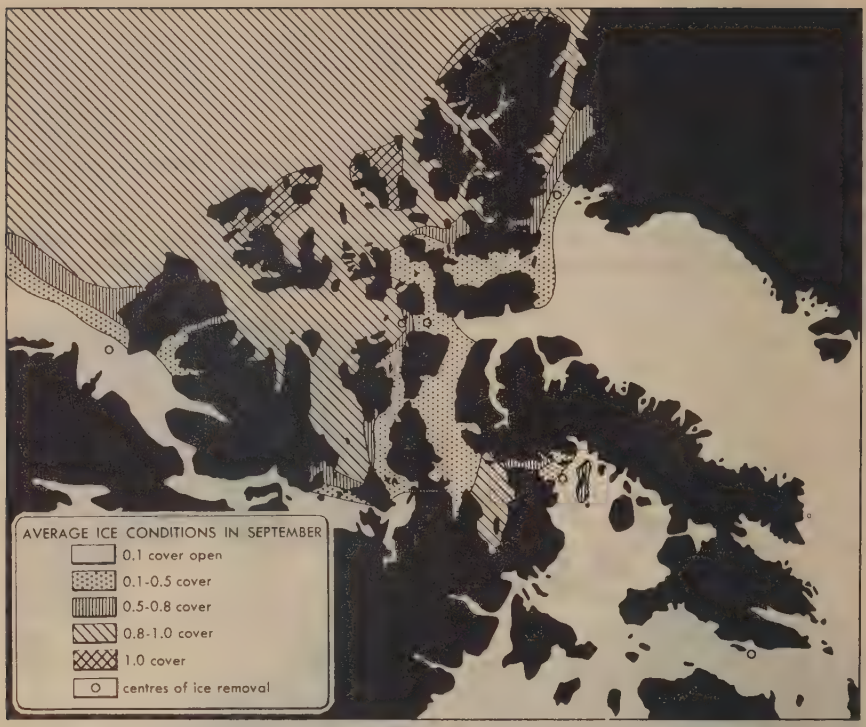

Fig. 3. September ice conditions.

end of Lancaster Sound where depths of 400 to 500 fathoms are common. In Lancaster Sound and Prince Regent Inlet the water depth varies between 100 and 300 fathoms, but to the westward the soundings decrease to about 200 fathoms in M'Clure Strait and Viscount Melville Sound. The sill depth through Barrow Strait is about 80 fathoms.

Few soundings have been taken in the channels in the northwestern part of the Archipelago or on the arctic continental shelf. The reconnaissance soundings indicate that the width of the shelf is approximately 50 miles off the coast of Banks Island and farther north increases to 100 miles. In the last year soundings of 150 to 200 fathoms have been recorded in the northern end of the Prince Gustaf Adolf Sea.

The distribution of sea-ice in the Canadian Arctic Archipelago is now fairly well documented and recently a sea-ice navigability atlas has been completed that will make available ice information in a form similar to that contained in ice atlases of the northern sea route [5].

Fig. 3 has been compiled from several sources including the Oceanographic Atlas of the Polar Seas, Part II [6] and the Ice Atlas of the Northern Hemisphere [7]. In several regions the distribution has been altered according to reports of observers who have been active in the Canadian Arctic, Grainger[8] and Learmonth (personal communication). The chart, which represents optimum ice conditions, shows that Lancaster Sound, Foxe Basin, and the passages south of Victoria Island are normally open, whereas in Prince Regent Inlet. Peel Sound, and Wellington Channel light ice concentrations are to be expected.

Recent analysis of ice records by Schule and Wittmann[9] has shown that

there are several areas where perennially unstable ice conditions can be expected. These areas form centres of ice removal and are indicated in Fig. 3 by circles.

Average conditions of temperature and salinity for the eastern and western Arctic are shown in Fig. 4. These curves have been constructed from observations taken in Lancaster Sound in 1957 and from the I.G.Y. drift station T-3, which in September 1958 was in the vicinity of the western end of M'Clure Strait.

Both curves show surface variations of temperatures and salinity caused by local sea ice. As would be expected surface temperatures and salinities are lower, 0°C and 29.90‰, in the Western Arctic since at the time the observations were taken the sea was totally covered with ice. In contrast, observations taken in the Beaufort Sea in September in areas of less than 5/10 ice cover show that under more comparable conditions very low salinities, and temperatures between zero and 6.0°C are characteristic of the surface waters to a depth of 30 metres [10].

The depth of the warm surface layer in Lancaster Sound indicates a prolonged period of surface heating and exchange with the warmer waters of the upper layers in Baffin Bay.

Similar temperature and salinity characteristics appear in both curves at about 180 metres but below this depth there is a uniform difference in both temperature and salinity that persists to the bottom. Bottom temperatures and salinity in the Western Arctic are almost constant at 0.4°C and 34.93‰; in the Eastern Arctic temperature and salinity at the bottom are typically 0.5°C and 34.50‰.

It is interesting to note that the slightly warmer interlayer at 75 metres that has been identified from the oceanographic observations taken from the Russian drifting station of 1950-51 is defined by temperatures of -0.7°C to -1.2°C. This feature does not appear in the 1958 T-3 records that show temperatures below -1.0°C from 10 to 180 metres; however, in 1952 Worthington[11] recognized a similar feature at positions in the same region as the Russian stations. At that time the negative thermal gradient between 50 and 150 metres was regarded as the last remnants of a seasonal thermocline. More recently Gudkovich[12] has interpreted this reversal of the thermal gradient as indicating the penetration of waters from the northern part of the Bering Sea into the arctic basin.

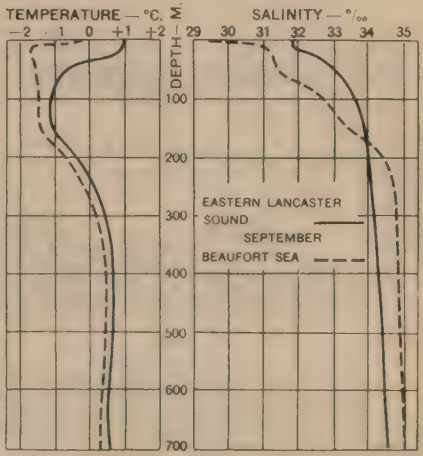

Fig. 4. Temperature and salinity curves.

The longitudinal profile through Parry Channel shown in Fig. 5 gives an idea of the east-west distribution of summer temperature and salinity in the Canadian Arctic Archipelago. In this diagram the bottom contour west of Barrow Strait is based upon very limited soundings and does not present an accurate picture of the bottom topography.

The typical sharp gradient of temperature and salinity in the surface layers is obvious and appears more pronounced in the west where heavy ice exists. Maximum surface temperatures of 2.5°C have been recorded in the eastern end of the section and the minimum surface temperatures of -1.6°C to -1.7°C normally appear in the region of Barrow Strait. The effect of the Barrow Strait sill is obvious in the discontinuity of the isopleths below a depth of 150 metres.

Temperature-salinity curves have been plotted in Fig. 6 for four representative stations in the Parry Channel system to show the variations in water characteristics from east to west. This graph shows the large variations in the surface layers and the pronounced differences in temperature and salinity that persist to a depth of 100 metres. Below 100 metres the curves assume a characteristic shape and can be considered representative for each specific region. temperatures of 0.4°C occur at about 500 metres and the salinity at depth is 34.93‰. These curves also show the similarity of the temperature-salinity characteristics of the deep water of Baffin Bay and the temperature and salinity features at depths of 250 metres in the arctic basin, an observation already discussed by Bailey[4].

Determinations of the concentration of dissolved oxygen in the water have been carried out on most of the recent

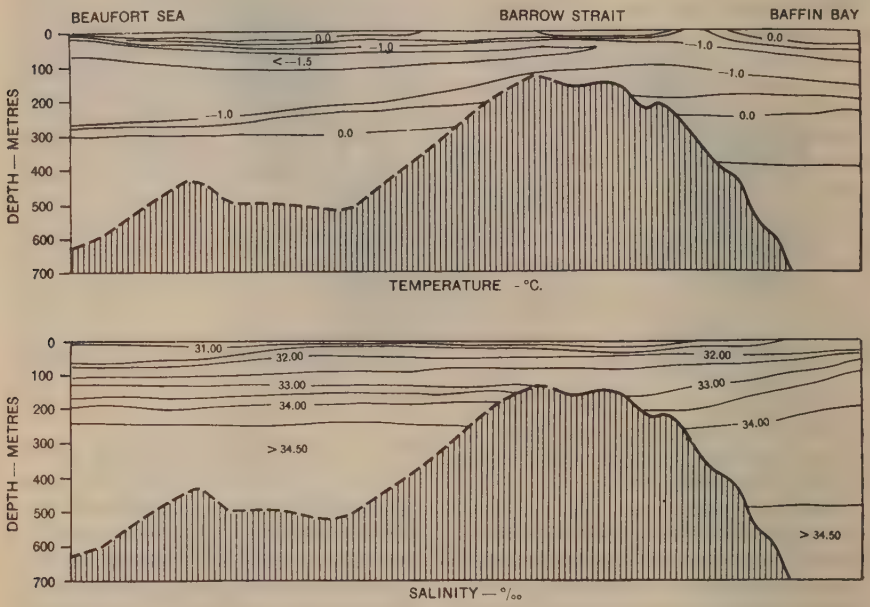

Fig. 5. Longitudinal profile of Parry Channel.

The variation of water characteristics above a depth of approximately 200 metres is apparent in the large spread of values in this depth interval and the east-west differences in the physical features below this depth are obvious in the displacement of the characteristic regional curves.

The graphs for the Eastern Arctic show an increase in temperature to a maximum of 0.8°C at 500 metres and a maximum salinity of 34.45‰ at the bottom. In the Western Arctic maximum oceanographic cruises in the North American Arctic. The observations that are confined to the summer months with few exceptions show that under similar conditions of ice cover there is little difference in the concentration of dissolved oxygen from east to west.

In eastern arctic waters where there are extensive ice-free areas and a constant exchange with adjoining basins the summer oxygen concentration varies from a maximum near the surface of 10.0 ml./l. to a minimum of 7.0 ml./l.

at 300 metres. In the Western Arctic under conditions of permanent ice cover a surface maximum of 10.0 ml./l. occurs after the period of peak surface run-off with a lag of about 1 or 2 weeks and the minimum of 6.0 ml./l. normally appears between 150 and 300 metres. Maximum

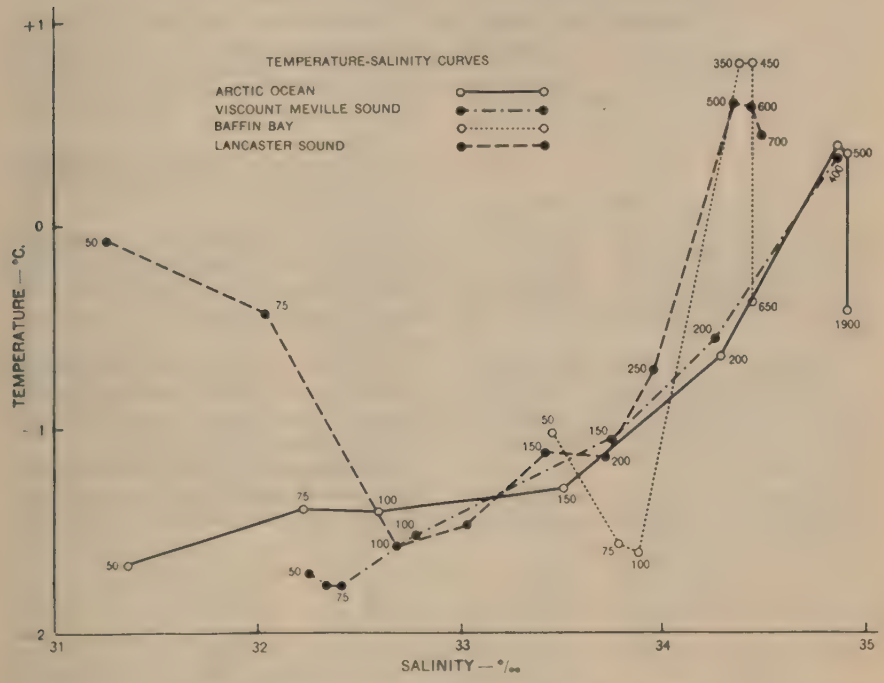

Fig. 6. Temperature and salinity in Parry Channel.

surface values of dissolved oxygen of over 10 ml./l. usually occur during August and the minimum content, as little as 5.0 ml./l., is found in September.

Calculations of the volume exchange through the channels of the Canadian Arctic Archipelago have been computed by several investigators including Kiilerich[1], Smith, Soule, and Mosby[2], and Bailey[10]. Recent surveys in Hudson Strait and Lancaster Sound by the Fisheries Research Board have determined the volume transport through these two major passages[13, 14].

A summary of recent information indicates that the total eastward transport of water from the Arctic Ocean through the Canadian Arctic Archipelago is of the order of 40,000 km.^{3} per year. Of this total approximately 16,000 km.^{3} per year pass eastward through Lancaster Sound and 13,000 km.^{3} per year enter Baffin Bay through Smith Sound. The remainder leaves the Arctic Ocean by way of Jones Sound and Hudson Strait.

Russian researchers have calculated the flow through the Canadian Arctic Archipelago based on the volume balance of the Arctic Ocean. Their results show a variation of values from 31,000 km.^{3} per year to the more recent figure of 8,000 km.^{3} per year [15, 16].

A. E. COLLIN

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More "Togetherness" Needed

Good refrigeration systems for transporting fresh or frozen fish are available but are not being used properly. The main obstacle is economics. To overcome this, at a price the consumer will pay, requires the cooperation of the shippers, transportation companies, and distributors.

When is the Canadian fishing industry going to make a serious effort to tackle this problem?

by O. C. YOUNG,
Assistant Chairman, Fisheries Research Board of Canada.

PERIODICALLY every industry and segment of industry may find it profitable to take a good look at itself, to assess its blessings, if pessimism is running high, to review its shortcomings, if optimism and self-complacency may be standing in the way of improvements. In this article an effort is made to describe some factors involved in the transportation of fish in both the unfrozen and frozen state, so that those interested in the transport business may consider these factors in relation to their own operations.

At the outset it should be observed that solutions are not promised. Indeed these appear to be so obscure in most cases, involving technology, mechanics and economics as they do, that one is inclined to become pessimistic, and is advised to "count blessings" to restore the optimism that is so essential in any effort to improve any situation.

Economics of Transportation

Dealing first of all with unfrozen fish: in the light of what is known about maintaining high quality in this form of commodity, one can, without hesitation, affirm that temperature and time are the principal factors to be considered in the transportation of fresh fish. The length of time that the commercially important species of fish can be safely stowed in crushed ice is fairly well known, as is also the holding time when bactericidal ices are employed. In addition, the "shelf life" of these species is known when chilled sea water and antibiotics are used. Since transportation is merely a modified holding or storage operation, one might wonder what remains to be solved in the matter of transporting fish when so much is known about holding them at high-quality levels. The answer, of course, lies in the economics of transportation.

Any innovation that involves other than the bare requirement of transporting objects from one place to another naturally results in added transportation costs, and if these additional costs ultimately result in reduced sales, then of course the innovation may be dropped or find only limited application. An example of this is the Canex container evolved by the Canadian National Railways some years ago. This container, through the use of cooling elements charged with a frozen eutectic solution which provided a container temperature a few tenths of a degree above the freezing point of fish muscle, gave excellent results in numerous trial shipments of dressed fish and fillets, but it did not come into general use because of the added costs involved.

In handling fresh fish, a highly perishable commodity, it must be emphasized that each operation in the continuum of events from the catching to the consumer's table, must receive its full share of attention. Fresh fish will have an ever decreasing quality related to time. Consequently if the time factor is known for each operation, such as for bringing the catch to port, unloading at the plant, inspection, packing, transportation, distribution, etc. and an acceptable quality target is predetermined, simple arithmetic will reveal just how long the fisherman will have to get his fish to the plant after the first capture. Unfortunately fishing operations are not carried on in this fashion.

Consumer wants Dividends, too!

In order that full advantage may be taken of each technological improvement related to the maintenance of high quality in fresh fish, to have fish take its proper place on the Canadian menu, it is important that each new principle be applied in such a way that every segment of the industry from the catcher to the consumer will shoulder its appropriate responsibility to receive its corresponding

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returns. That is to say, if the application of a new principle will prolong the shelf life of fish a given number of days, the consumer should share in the dividends, along with the fisherman and others, by getting higher quality fish.

This involves a togetherness that does not always exist, of course, and is somewhat idealistic. Nevertheless it does contain the elements of a solution to the transportation problem which is largely economic as already explained. The transportation companies, given more time and higher quality fish, presumably could deliver better fish by treating shipments with the same urgency as in the past, and the consumer reaction to the higher quality could be such that better transportation techniques such as the Canex container would become economically feasible. In short, "togetherness" could be the solution to the problems associated with the transport of fresh fish.

Turning now to the transport of frozen fishery products, here again the problems are similar to those involved in storage with the only added requirement of mobility.

For the maintenance of high quality in frozen fishery products, it is now generally agreed that sub zero (Fahrenheit) temperatures are necessary for prolonged storage periods. Recent biochemical studies and taste panel assessments have shown also that *constant* temperatures are important, particularly if variations involve temperatures rising to the neighborhood of 15 deg. F. such as might occur when shipments are made in mid-summer weather.

Fisheries Research Board and other scientists have made extensive studies of the effects of freezing and subsequent cold storage on fish muscle proteins. They have adduced much evidence to show that undesirable changes take place, particularly at temperatures above zero. Certain proteins are denatured and fats are oxidized. The rates of these actions are dependent upon temperature level and constancy of temperature. Since these factors are being coped with in the modern cold storage plant, cannot they be handled in the transportation phase. Technically speaking the answer is in the affirmative, but the necessity for mobility imposes a condition that makes the holding of optimal storage conditions economically difficult.

The reader will probably remember the early work done by the Fisheries Research Board in collaboration with the Canadian National and the Canadian Pacific Railway Companies which culminated in the adoption of the overhead-cooled refrigerator car as the standard in Canada. Subsequent technological advances showed that temperatures obtainable in such cars were not satisfactory in midsummer weather when using ice and salt for cooling, so mixtures with lower melting points were tested by the National Research Council with some success. In addition, mechanical refrigeration was experimented with by the Fisheries Research Board and the two above Canadian Railways, employing systems still being tested in the United States also with some success. None of these systems, however, have proven themselves economically yet in Canada.

Refrigerated Trucks fail Test

It was thought that the larger trailer trucks equipped with "packaged" type automatic refrigeration units were providing a partial answer to the frozen transport problem, because they offered quick service and presumably provided specified holding conditions. Unfortunately, controlled tests made recently by the National Research Council in collaboration with the Department of Fisheries have shown that trucks are not regularly meeting the desired target of 0 deg. F. by a substantial margin. Analyses of the test data revealed that in some cases the insulation or the refrigeration units were inadequate, while in others the operation was at fault. Further investigations are planned for 1961 by the same collaborating organizations.

Liquid Nitrogen Process

A more recent interesting development is the use of liquid nitrogen for the shipment of frozen products. In the experiments so far conducted, the liquid nitrogen was employed at atmospheric pressure, thus involving a temperature of -320 deg. F. Since nitrogen is inert and is present in our atmosphere to the extent of 79 per cent, it certainly can be safely used in direct contact with foods. Indeed it could have a desirably depressing effect on oxidation, by eliminating oxygen from contact with commodities during shipment.

With a refrigerant at a temperature as low as -320 deg. F. there is no question that the target of 0 deg. F. or lower may be reached with ease, and this has

The Liquid Nitrogen Process — This new refrigeration development can keep fishery products at a very low temperature for impressive periods of time. Here a Lique-Freeze container is shown being frozen down to minus 320 degrees Fahrenheit.

been demonstrated in some experimental shipments. Furthermore we are told that a liquid-nitrogen system can be installed in a truck or railway refrigerator car relatively cheaply and because precooling is not necessary and the system makes possible other savings, it is held to be a potential competitor as a source of refrigeration in the transportation field. Technically, it could be a definite contender for first choice, but again economics will determine what the future for it will be.

This is a fast-moving age, and our advances are not only in the fields of nuclear physics, rockets and machines. Ever increasing efforts are being directed to studies on nutrition and the effects of eating habits on organic ailments afflicting man. Quality standards for foods are under constant review by such organizations as the Department of Fisheries Inspection Branch and the Department of National Health and Welfare in Canada, and the U.S. Bureau of Commercial Fisheries and the Association of Food and Drug officials of the United States. This latter Association has developed a code covering, among other things, the holding temperature for frozen foods. Since Canada has substantial markets in the United States for much of its frozen production, particularly fishery products, it is very important that weaknesses in our transportation facilities be given immediate attention.

The present indications are that the obstacles to the adoption of technically feasible refrigeration systems at the moment lie in economics. That does not mean that other aspects of the problems need no further consideration. If the transportation companies in Canada do not soon come up with conditions acceptable to shippers and distributors, Canadians may follow the trend in the United States where, we are told, frozen food packers to some extent are operating their own refrigerated trucks.

Again it would seem that a solution involves a high degree of togetherness, to the end that the most promising features of all known elements, be they technical or economic, are carefully studied together with the primary purpose of meeting the optimal requirements for the foods themselves, at a price that the consumer will pay.

* * *

Investigation and Management of Atlantic Salmon

The Research Programme

By C.J. KERSWILL

Fisheries Research Board of Canada,
Biological Station, St. Andrews, N.B.

Summary of 1960-61 Reports

THE EXTENSIVE programme of investigation and management of Atlantic salmon in Eastern Canada includes research activities by biological stations of the Fisheries Research Board of Canada at St. Andrews, N.B., and St. John's, Newfoundland, by the Province of Quebec, and by the Canadian Wildlife Service of the Department of Northern Affairs and National Resources. Co-operation in related management programmes that depend on research is maintained with many organizations including the Fish Culture Development and Protection Branches of the federal Department of Fisheries, and various departments and groups concerned with salmon fishing in the Atlantic Provinces.

In 1949 co-ordination of salmon research and management was assigned to a Federal-Provincial Co-ordinating Committee on Atlantic Salmon. In 1959 the responsibility was taken over by a new Federal-Provincial Atlantic Fisheries Committee, which has a Salmon and Trout Section and under it a Scientific Advisory Group and an Industry Advisory Group. Progress with existing projects and plans for future work are reviewed annually at a meeting of the Scientific Advisory Group. The following report is based on 1960-61 reports presented at the last meeting, held at Halifax, N.S., on March 8 to 10, 1961, under the chairmanship of Dr. J.L. Hart, Director of the Biological Station, St. Andrews,

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N.B. Reports on major topics of investigation were received from Drs. P.F. Elson, M.H.A. Keenleyside, J.B. Sprague and C.J. Kerswill of St. Andrews, N.B.; Mr. A.R. Murray, St. John's, Newfoundland; Messrs. R. Bourassa and L. Tremblay, Quebec; and Mr. A.J. Erskine, Canadian Wildlife Service, Sackville, N.B.

Again our salmon investigators wish to thank many people for their help, including administrative officials of various federal and provincial government departments, fishery officers, fish culture staff, technicians, seasonal assistants and commercial fishermen's and anglers' associations. Without their constant co-operation little could be accomplished in our efforts to improve management of the commercial and sport fisheries.

First it may be helpful to outline the various salmon stages and the names commonly used for them. Adult salmon spawn in the autumn, burying their eggs wherever suitable gravel occurs between head of tide and the uppermost reaches of rivers. In spring the young emerge from the gravel as fry, and are called underyearlings during their first year. Older young salmon are called parr. The young fish usually stay in the rivers for two to four years but sometimes longer, after which they migrate to sea in spring as smolts after growing to a length of at least four inches. The adults may return to fresh water as grilse weighing up to six pounds after one winter at sea, or as larger salmon weighing about eight pounds or more after an additional year or longer at sea. Some adults enter fresh water in the spring, others do not enter until autumn, but all wait until October to spawn. Only a small fraction, usually under 10 per cent, of fish that have spawned once survive to return for further spawning. In the Maritime Provinces, salmon commonly need six years to complete one full life cycle, but there is considerable variation among different areas.

AVAILABILITY OF ADULT SALMON

(1) Maritime catch statistics

The Department of Fisheries obtains records of salmon caught by commercial nets and angling in the Maritime Provinces. Yearly totals for the 1949-60 period are shown in Figure 1. The "Maritime Region" has three areas, "Gulf", "Atlantic" and "Fundy", chosen to include rivers producing salmon with similar life-histories. The Gulf Area extends from Cape Gaspe, Quebec, around the Gulf of St. Lawrence to Fourchu, Cape Breton Island. The Atlantic Area extends from Fourchu around the outer coast of Nova Scotia to Cape Sable. The Fundy Area takes in the Bay of Fundy coasts of Nova Scotia and New Brunswick. Commercial landings for the Gaspe, Quebec, part of the Gulf Area were obtained from reports by the Quebec Bureau of Statistics.

Graph A shows that total commercial landings in 1960 fell about 10 per cent below the 1959 level, mainly because of a lower catch in the Gulf Area. The latter was expected because grilse (one-sea-year fish) were scarce in 1959, as a predicted result of DDT sprayings in 1956 and 1957, and the 1960 commercial catch (mostly two-sea-year fish) depended on the same year-class. Low river outflows in the latter part of the commercial season also contributed to poor catches in estuarial trap nets.

Salmon angler, Northwest Miramichi River, N.B.

Graph B shows that 1960 angling catches in all areas continued a downward trend since the last peak in 1958. Much of the Gulf Area decrease was likely the predicted effect of DDT sprayings on young salmon of the Southwest Miramichi watershed. Few salmon were available there to anglers during the early part of the season, although river levels were near normal for that time of year because of a prolonged spring freshet. Early angling in the Northwest Miramichi was affected by mine pollution. In midsummer, low-water conditions interfered with angling throughout the Miramichi, but there was improvement in September. Severely low water levels in Nova Scotia rivers likely accounted for the 1960 catch being one of the three lowest in the Atlantic Area since 1949.

(2) Quebec and Newfoundland statistics

In 1960 commercial landings in the Province of Quebec totalled 0.59 million pounds, almost equal to the 0.60 million pounds landed in 1959. Angling statistics for Quebec are not available.

In Newfoundland commercial landings fell by 12 per cent from 2.34 million pounds in 1959 to 2.07

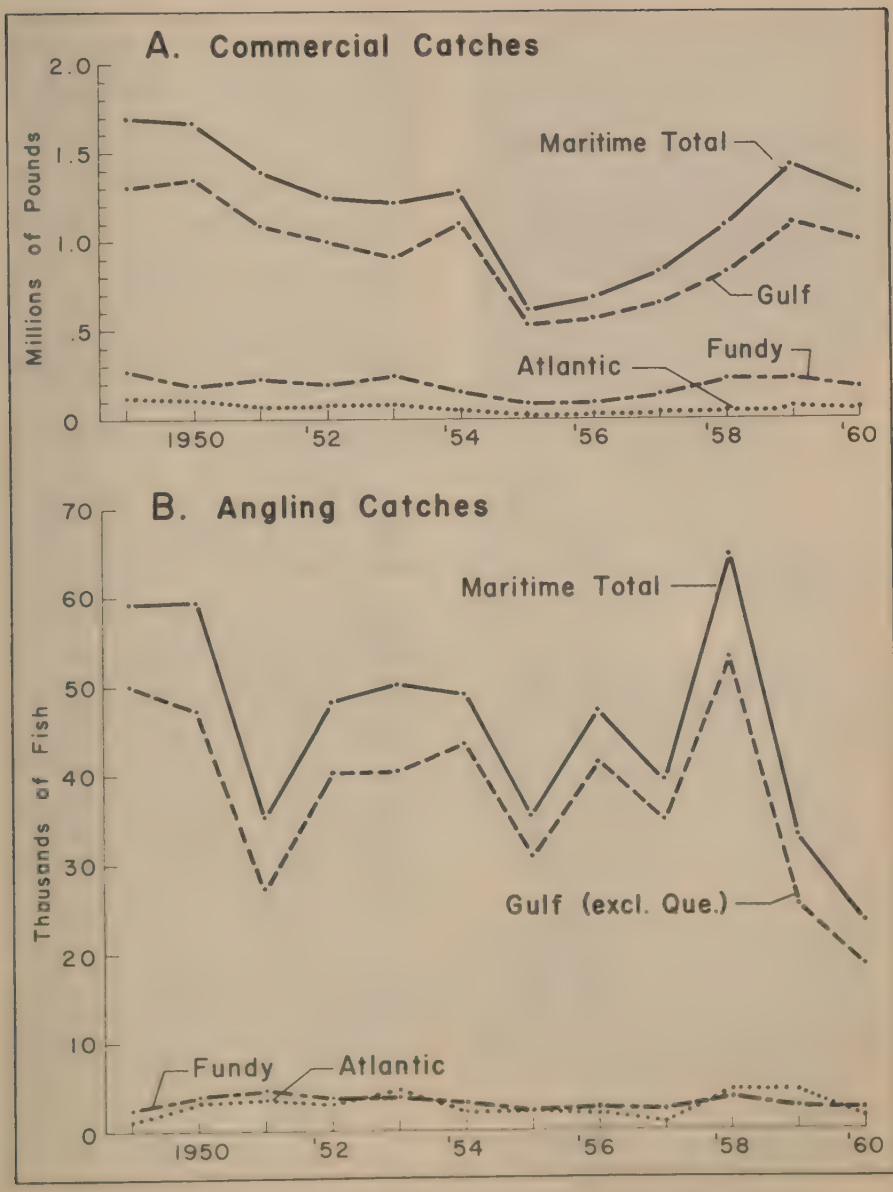


Figure 1. Atlantic salmon catches in Maritime Region, Canada, 1949-60.

million pounds in 1960. In 1960 angling catches in Newfoundland rivers totalled 18,361 fish as compared to 19,814 in 1959, a decrease of only seven per cent; angling effort decreased from 37,071 rod-days in 1959 to 36,384 rod-days in 1960, or by two per cent.

(3) Comparison of Maritime angling catches, 1958-1960

Table I compares data of the past three years on angling catches and fishing effort in rod-days in the Maritime Region, its three areas, and some individual rivers. The third, sixth and ninth columns show average success per 10 days of angling.

(4) Counting fences and sampling traps

The Fisheries Research Board continued to record the number of adult salmon ascending the Pollett River, N.B., the Northwest Miramichi River, N.B., at two points, and the Little Codroy River, Nfld. For the seventh year a sampling trap similar to a commercial trap net was operated near Chatham in the Miramichi estuary through the open-water season. As usual some fish were tagged to show their movements to other parts of the river system. The data are a useful addition to catch statistics in showing the time and size of adult runs before, during and after the public fishing seasons. Also, they indicate the effects on salmon abundance of varying weather and water conditions, and of changes resulting from forestry, mining and other man-made conditions.

The Miramichi traps have provided the following general picture of adult salmon movements through the estuary and up the Northwest Miramichi tributary. Grilse: From 20 to 40 per cent of the total annual run pass through the estuary during the commercial season from June through August; the balance ascend later. The total run reaching Cur-

Table I. Comparison of angling catches of salmon in 1958, 1959 and 1960, Maritimes Region

	1958			1959			1960		
	No. fish (C)	Effort rod-days (E)	$\frac{C}{E}$ x 10	No. fish (C)	Effort rod-days (E)	$\frac{C}{E}$ x 10	No. fish (C)	Effort rod-days (E)	$\frac{C}{E}$ x 10
Maritime Region Total	65,048	157,848	4.1	33,030	124,727	2.6	23,495	109,502	2.1
Gulf Area Total	56,764	74,383	7.6	25,499	56,368	4.5	18,748	46,427	4.0
Miramichi System	45,067	58,135	7.8	20,531	42,108	4.9	14,064	34,142	4.1
S.W. Miramichi	34,164	38,901	8.8	12,034	25,478	4.7	10,445	24,351	4.3
N.W. Miramichi	3,147	6,489	4.8	4,617	6,496	7.1	1,707	3,987	4.3
Little S.W. Miramichi	3,241	5,845	5.5	1,599	5,600	2.9	1,385	4,195	3.3
Renous	1,502	3,061	4.9	475	1,260	3.8	230	1,049	2.2
Cains	2,763	3,270	8.4	1,691	2,651	6.4	297	560	5.3
Restigouche System	9,268	2,412	38.0	3,396	6,077	5.6	3,102	4,991	6.2
Restigouche	7,012	1,648	42.5	2,824	3,699	7.6	2,278	3,555	6.4
Kedgwick	356	372	9.5	144	698	2.1	151	406	3.7
Upsalquitch	1,900	392	48.4	428	1,680	2.5	673	1,030	6.5
Nipisiquit	1,048	3,540	3.0	461	2,150	2.2	243	905	2.7
Tabusintac	576	4,796	1.2	605	1,845	3.3	717	1,730	4.1
Margaree	334	1,275	2.6	235	1,110	2.1	140	1,050	1.3
Atlantic Area Total	4,500	66,468	0.7	4,564	47,999	1.0	1,909	46,039	0.4
St. Marys	735	4,392	1.7	550	2,970	1.9	278	2,284	1.2
Moser	207	6,475	0.3	392	4,030	1.0	207	7,635	0.3
Sheet Harbour	368	14,945	0.2	455	8,160	0.6	167	9,715	0.2
LaHave	807	2,265	3.6	639	2,005	3.2	81	1,347	0.6
Medway	1,038	7,668	1.4	1,023	9,995	1.0	551	8,468	0.7
Fundy Area Total	3,784	16,997	2.2	2,967	20,360	1.5	2,838	17,036	1.7
Saint John (main)	2,518	6,401	3.9	1,753	9,116	1.9	1,827	7,226	2.5
Nashwaak	218	3,021	0.7	195	2,991	0.7	88	1,736	0.5
Tobique	186	539	3.5	355	650	5.5	377	1,190	3.2
Petitcodiac System	337	1,250	2.7	247	1,115	2.2	166	1,110	1.5
Salmon (St. John County)	161	1,535	1.0	44	1,015	0.4	238	1,476	1.6

venton, seven miles above tide-head on the Northwest Miramichi, averages about 2,000 per year. At least 80 per cent of them arrive during the angling season from May through September. Many of them reach Camp Adams, 33 miles farther upstream, during the angling season. Large salmon: Several hundred are taken annually in the estuarial sampling trap during the commercial season, as in nearby public traps. About ten times as many are counted through the trap from September into early November. The total run of large salmon at Curventon averages about 1,000 per year. From 10 to 80 per cent of them pass Curventon before September 30, depending on whether or not there are few or many freshets during the summer. Less than 30 per cent of all the large salmon passing Curventon reach Camp Adams, 33 miles farther upstream.

DISTRIBUTION AND USE OF SALMON OF KNOWN RIVER ORIGIN

Marking descending smolts by fin-clipping was continued on the Pollett and Miramichi rivers for later recognition of the fish as adult salmon in fisheries and at counting traps. On the Miramichi, Swedish-type smolt tags were applied to part of the downstream migrants for the third successive year. At the Pollett River, 1,000 new dart-type tags were applied to young hatchery-reared salmon planted in October, and it is hoped that future recaptures of tagged smolts and adults will show the usefulness of the technique.

Of the 1,000 Miramichi smolts tagged in 1958, one was taken as a grilse in 1959 by a Miramichi angler; six were recaptured as large salmon in 1960, of which two were caught by Miramichi anglers, two were taken in commercial traps in Margaree Harbour, N.S., and Bay Chaleur, and two by Miramichi drift-nets. Of 3,500 Miramichi smolts tagged in 1959, 24 were recaptured as grilse in 1960; of these, one taken in commercial gear off Greenland in October and one taken in the upper Bay of Fundy in June were of special interest. It is hoped that a good number of the 1959 tags will be recovered on two-sea-year salmon during 1961. As usual, many recaptures of adults that had been fin-clipped in previous years on the Miramichi and Pollett Rivers were made in commercial gear over a wide area of the Canadian Atlantic coast, and in fresh water of the home streams.

The Little Codroy, Nfld., project is providing useful information on the relationship between the number of smolts which migrate to sea and the number of resulting adults. Annual smolt production has averaged about 12,000, and from two to three per cent can be accounted for as adults, including those caught in commercial fisheries, and those returning to the river of origin.

YOUNG SALMON PRODUCTION

1. Pollet River experiments

Experiments during the past 18 years have

Salmon drift-net boats, Escuminac, N.B., June, 1960.

clarified views on the factors that control natural production of salmon up to the smolt stage, and have indicated practical methods of overcoming smolt deficiencies. The results of many projects have been reported, for example, the beneficial effects of predatory bird control, suitable ways to use hatchery stocks in planting, and the number of spawners needed for optimum natural smolt production. Management principles developed on the Pollett are now being tested and further developed on other rivers.

Analysis of six years of population data on young salmon and other fishes obtained at a large series of sampling sites indicates the relative suitability of different types of stream bottom as salmon habitats. More young salmon are found per unit area in water under 10 inches in depth than in deeper water, and more occur in rapids than in flats and pools. Parr are about equally abundant over gravel, cobble or rock bottom, but many more fry occur over gravel than over other bottom types.

2. Young salmon assessments, Maritime Provinces

In 1960 autumn electroseining was continued at a large number of stations on representative areas of the Pollett, Miramichi, Tobique and Margaree rivers, and extended to the Nashwaak. It is an essential part of studies of the effects of DDT sprayings, experimental bird control, hydroelectric development and mine pollution. It gives a direct measure of the numbers of different year-classes of fish per unit area, and is the best available method of assessing effects of environmental conditions on smolt production.

ENVIRONMENTAL CHANGES

DDT spraying

Aerial spraying of DDT to control a spruce

budworm outbreak in the Maritime Provinces commenced on a small scale in 1952 on 186,000 acres of woodland in northern New Brunswick. From 1953 to 1956 between 1.5 million and 2.5 million acres were sprayed annually in New Brunswick and the Gaspe Peninsula, Quebec. As the outbreak spread in New Brunswick to the east and south, it soon covered the watersheds of the Upsalquich, Nipisiquit, and Miramichi rivers, and parts of the Restigouche and Saint John watersheds. In 1957, 6.5 million acres were sprayed, including five million acres in New Brunswick, over most of the Restigouche, Nipisiquit, Tobique, and Miramichi watersheds; some areas were sprayed for the second or third time. In 1958, 2.6 million acres were sprayed in New Brunswick, mainly to the west and south of earlier operations over the Saint John River. In 1959 no commercial spraying occurred and it was thought that the outbreak had collapsed. But a resurgence occurred in the southern part of the previously sprayed area and over two million acres were sprayed in central New Brunswick in 1960, affecting the Saint John and the southern part of the Miramichi. A similar area was scheduled for spraying in 1961. Forest biologists have attributed the recent collapse of the outbreak in the northern areas to recovery of natural controls, and the southern resurgence to particularly favourable weather conditions for budworms.

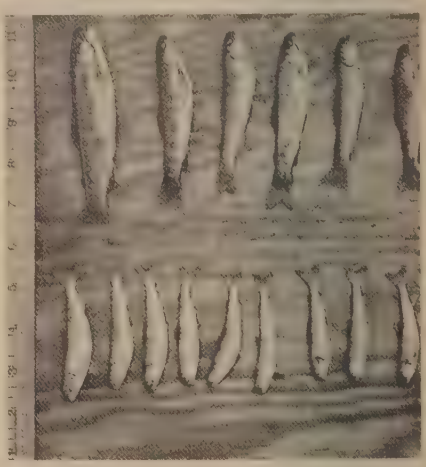
Annually since 1954 the salmon research staff of the St. Andrews Station has put much effort on the insecticide-fish problem in New Brunswick. At first it was aimed at learning how much effect the regular operational spraying in 1954 had on the survival of young salmon in the affected streams, and on the stream organisms that are their chief food. By 1956 the effects on returning adult salmon could be observed at Miramichi sampling traps and counting fences. In 1958 and 1959 the effects of various strengths of DDT insecticide on young salmon and trout, and on stream insects were tested during small-scale experimental spray operations.

The tests in 1958 and 1959, carried out jointly with the Forest Biology Division, Department of Agriculture, indicated that application of 1/4 pound of DDT per acre had less serious immediate effects on fish and their food than the usual 1/2 pound of DDT per acre. The lower concentration gave adequate budworm control, if enough small droplets of the insecticide reached the tree needles. In 1960 similar field checks were made in the two million acre operational area, to compare effects of full-strength and half-strength insecticide on salmon and trout as well as their food. It was possible because the Saint John River drainage was scheduled to receive full-strength spray, while the Miramichi drainage was to receive lower concentration, mostly half-strength.

The fisheries part of the 1960 programme included observations on the following.

1. Caged young salmon and trout at six places, mainly by Department of Fisheries staff with supervision provided by the Board. By June 18, eight days or longer after spraying, there

Checking on survival of young salmon and trout held in cages in Cross Creek, N.B., sprayed with full-strength DDT insecticide, 1960.



Some of the caged young trout (above) and young salmon (below), that died within 3 days of spraying by full strength DDT insecticide in June, 1960.

were considerable differences in survival in full-strength and half-strength areas, with minimum losses in unsprayed controls. For example, percentage losses of salmon parr averaged: full-strength, 78 per cent; half-strength, 38 per cent; control, 17 per cent.

2. Stream invertebrates by bottom sampling at eight stations before and after spraying, and collecting drifting organisms on small vertical screens. At all check points reductions that could seriously affect the diets of young salmon and trout occurred within several days of spraying. In the unsprayed control stream there was no evidence of similar effects and,

Collecting stream invertebrates from bottom sampler, in study of effects of DDT spraying, Miramichi area, 1960.

in fact, increases usually occurred through hatching of young. Water analyses for DDT content by Department of Agriculture staff at short intervals following spraying indicated that the insecticide settles out of the water within a few hours. The relationship between kill of stream invertebrates and concentration of DDT in the water is to be studied in detail during 1961.

3. Native young salmon by electroseining census. This has always been regarded as the most useful indicator of the effects of full-scale insecticide sprayings on salmon. In 1954, when spraying first occurred in the Miramichi watershed, the seriousness of its effects was suggested by the deaths of young salmon held in cages and by the many dead young salmon, trout and other fish found along stream banks within several days of spraying. But proof of the extent of effects of the June 1954 spraying on native young salmon living normally in the streams came from the regular annual census by electroseining during August and September 1954. Data have been obtained in a similar manner annually since 1950 at a large number of seining stations in the Northwest Miramichi, Renous, and Cains tributaries. The data have formed the basis of predictions of effects of DDT spraying on adult runs. In 1960 additional seining stations were included on the upper Southwest Miramichi and the Nashwaak to help compare effects of full-strength and half-strength sprayings. The 1960 autumn census showed that with 1/2 pound DDT per acre, underyearling salmon are almost eliminated in the year of spraying; 1/4 pound DDT per acre leaves from one third to one half the normal number of underyearlings. Older parr also appeared to have better survival with lighter DDT concentration; for example, in the Cains River, sprayed at 1/4 pound DDT per acre, over 90 per cent of the fish that were small parr in 1959 survived to become large parr by autumn 1960.

4. Late-season losses. In previous years, by autumn in the year of spraying, surviving young salmon were relatively thin. As the river water approached freezing temperatures, additional deaths were observed frequently in areas subjected to full-strength spraying, but not in unsprayed streams. In October 1960, samples of 100 live parr were collected by electroseining in streams within the full-strength and half-strength areas, as well as in unsprayed areas, and taken to the St. Andrews laboratory. They were held under similar conditions in tanks, and some were chilled to simulate onset of cold-water conditions in streams. From October 5 to November 10, while the water temperature fell from 57°F. to 53°F., 40 per cent of the Nashwaak (full-strength DDT) died, or three times as many as from the half-strength and unsprayed streams. Quick chilling to near freezing temperature and holding at 33°F. were followed by over 80 per cent loss of full-strength sprayed fish within eight days, but no half-strength sprayed or unsprayed fish died during a month.

5. Recovery of aquatic insects in areas sprayed, 1954-57. Parts of the Northwest Miramichi watershed were sprayed with 1/2 pound DDT per acre in June of 1954, 1956 and 1957. Millstream Brook, a nearby stream with similar characteristics, has never been sprayed. Annually since 1955 the production of aquatic insects has been assessed from May to September by collecting adults emerging into sev-

eral series of yard-square cage-traps at 24-hour intervals five times a week, in the sprayed and unsprayed areas. The 1960 data show that good recovery has occurred among all groups of insects with the exception of caddisflies, which are an important item of the diet of large parr.

Mining pollution, Northwest Miramichi River

In 1960 evidence was obtained that mining pollution of the Tomogonops River, which enters the Northwest Miramichi 15 miles above Curventon, was seriously affecting both young and adult salmon of the Northwest Miramichi. Counting-fence records at Curventon showed that 24 per cent of the grilse and 10 per cent of the large salmon ascending from June to November came back down. Highest percentages recorded in six previous years of observation were four per cent for grilse and two per cent for large salmon. Upstream migration to the upper counting fence at Camp Adams was delayed, and few fish reached upper areas of the Northwest Miramichi during the angling season. Recaptures of salmon tagged while ascending at Curventon showed that some were caught in other branches of the Miramichi system after passing downstream.

Caged salmon parr held in the Tomogonops outflow in July all died within 90 minutes. In August, resident young salmon were scarce for several miles below the mouth of Tomogonops, as compared to populations there in previous years and in the Northwest Miramichi above the outflow in 1960. The food of young salmon was seriously affected; all groups of invertebrates were reduced in Tomogonops water and mayflies were affected drastically. The few young salmon remaining in the affected part of the Northwest Miramichi made poorer than normal growth during the summer.

The evidence of harmful effects provided by the Fisheries Research Board data resulted in legal action to lessen pollution in 1960 and prevent it thereafter.

This additional and unexpected local change in the environment of salmon confused the research situation on the Northwest Miramichi insofar as getting further evidence of effects of earlier DDT sprayings on runs of adults. It did provide, however, an unique opportunity to acquire much new information on the effects of pollution by zinc and copper on Atlantic salmon.

BEHAVIOUR STUDIES

Studies of the behaviour of young salmon were continued in observation tanks at the St. Andrews laboratory and under natural conditions in the Miramichi River. Detailed analyses were made of various activities associated with the holding of territories by individuals and groups on the stream bottom. Such fundamental information can have practical significance in salmon management. For example, the fate of hatchery-reared fish planted in rivers may depend on their ability to secure and hold territories in competition with wild fish.

Fish Liver Paste

Properly Prepared, It Can Be Tasty, Nutritious Food

By A. GUTTMANN

Dr. Guttmann is on the staff of the Fisheries Research Board of Canada at its Technological Station, Halifax, N.S. He is there on loan from the Nova Scotia Research Foundation.

THE PURPOSE of this article is to attract attention to the high nutritive value of fish livers. Much work has been done in the Halifax, N.S., Technological Station of the Fisheries Research Board of Canada and also in laboratories abroad to prove that fish liver does not differ much from mammalian liver in the content of essential vitamins and could replace mammalian liver as a food supplement.

Table I lists the qualities of some inorganic and organic compounds present in both. These figures also show that fish livers compare favourably with pork liver. Moreover the presence of certain growth factors in fish livers which are chemically not determinable was demonstrated. In addition the fat contains large proportions of unsaturated fatty acids which may add some nutritional value to this product.

People supposedly do not buy cod or haddock livers at the fish market because, unlike beef or pork liver, they contain, according to size and season, 35 to 65 per cent oil. The fish oil industry, which processes thousands of tons annually, has enormous quantities of residue on hand which could be used for human consumption. There is no doubt that fish liver paste, properly prepared, could be used as a sandwich spread and could achieve, with time, the same popularity as sardines, and perhaps greater, because of its higher nutritive value.

In this laboratory fish liver pastes were made from cod and haddock livers with different seasonings. After canning, they were kept at room temperature for more than two years. During this storage, no change in appearance or taste was noted.

PREPARATION OF FISH LIVER PASTE

Fish livers which are to be used for liver paste should be fresh and have their original shape. Any tissues attached to them, particularly the gall bladder, should be removed. After a slight rinsing with cold water, they are ground. For this purpose a large Waring blendor was used in this laboratory, but any grinding machine could be used if properly cleaned before use. The oil was separated by the procedure of F.A. Vandenheuvel, (Atlantic Progress Report, No. 48, pp. 4-7, 1950; U.S. Patent 2,588,338, Mar. 11, 1952 and Canadian Patent 484,092, June 17, 1952) in which alkali is used in a quantity just sufficient to raise the pH from 6.8 to 8.5 or 9.0 and the temperature raised to 60 to 65°C. The Alkali is added under vigorous stirring when

Table I. Inorganic and organic constituents of enzymic hydrolysates, calculated on a water-free basis (Journal of the Fisheries Research Board of Canada, 12: 637-645, 1955).

Enzymic hydrolysates of	Calcium	Total phosphorus	Copper	Iron	Total nitrogen	Choline	Thiamine	Riboflavin	Niacin	Pantothenic acid	Vitamin B 12
	%	%	ppm	ppm	%	%	ppm	ppm	ppm	ppm	ppm
Cod liver residue	0.085	2.03	78	158	12.87	1.26	2.95	101.0	369	89	3.80
Haddock liver residue	---	---	79	160	11.05	2.24	1.61	73.0	321	57	0.86
Salmon liver, dried and defatted	0.092	1.49	---	---	12.60	1.31	8.90	61.0	262	79	4.80
Pork liver, dried and defatted	0.063	0.86	150	167	12.30	1.86	4.48	131.0	616	231	1.35
Beef liver, dried and defatted	0.049	1.04	178	74	11.10	1.75	3.92	138.0	888	261	4.79

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the correct temperature is reached. Stirring is continued for about five minutes while the oil separates. The oil is removed by centrifuge and the residue, which has been exposed to the elevated pH and temperature for only a short time is immediately neutralized with dilute hydrochloric acid to pH 6.8.

Example. 100 kg. (220 lb) minced fresh cod livers were brought while stirring to 60°C (140°F). At this temperature 833 ml (1.5 pints) of a 28 per cent sodium hydroxide solution was added under vigorous stirring. Stirring was continued for about five minutes and then the mixture was centrifuged. The amount of oil obtained was 59.6 kg (130 lb) and the weight of the residue was 40 kg (88 lb). To restore the residue to its original pH, 1200 ml (2.11 pints) of dilute hydrochloric acid was added (400 g HCl sp. gr. 1.18 - 1.19 add water to 1 liter (1.76 pints). The figures mentioned for sodium hydroxide and for hydrochloric acid were always correct when the livers were really fresh. When the oil separation required a greater amount of alkali than indicated, the residue could not be used to produce a good liver paste. In that event, the liver had not been fresh enough and free fatty acids had been formed by enzyme activity.

To prepare the paste, 100 kg (220 lb) of neutralized residue, 6 kg (13.2 lb) of sieved wheat flour and 3 kg (6.6 lb) of vegetable oil were mixed; salt and spices were added.

The kind and amount of salt and spices to be added are a matter of taste. The following quantities were used for 100 kg (100,000 g; 220 lb) of residue:

Common salt	1200 g)		(2 lb 10.5 oz)
Celery salt	775 g)	2750 g	(6 lb 1.7 oz)
Onion salt	775 g)		(1 lb 11.4 oz)
Black pepper	9 g		(0.32 oz)
Mace	5 g		
Garlic	5 g		(0.17 oz)
Nutmeg	5 g		
Thyme	5 g		
Bay leaves	5 g		
Curry	150 g		(5.3 oz)

The resulting paste was generally accepted and considered very palatable.

Onion salt and celery salt were prepared by mixing the minced vegetables with salt (in a ratio 1:10) and sieving when air dry.

To prevent uneven mixing of the small amounts of the different spices with a large quantity of paste, it was found advantageous to use alcoholic extracts of the spices instead of the dry powders. The concentration of the extract found suitable was 25 to 30 per cent.

Reprinted from
Proceedings of the
GULF AND CARIBBEAN FISHERIES INSTITUTE
Thirteenth Annual Session, November, 1960
Pages 109-112

Rehabilitation of Disease-Depleted Oyster Populations in Eastern Canada

R. R. Logie[1], R. E. Drinnan[2] and E. B. Henderson[2]

INTRODUCTION

In Eastern Canada, oysters, *Crassostrea virginica*, Gmelin, occur in those portions of the provinces of Prince Edward Island, New Brunswick and Nova Scotia bordering the southwestern Gulf of St. Lawrence.

In 1915 oysters started dying in large numbers in Malpeque Bay, Prince Edward Island. Mortalities fanned out from an apparent epidemic centre and soon involved Cascumpeque and Bedeque Bays. By 1920 the fishery of these waters was prostrate. Subsequent investigation, ten or more years after the subsidence of spectacular mortality, suggested:

(1) that in infectious, epidemic disease was involved;

(2) that mortalities exceeded 90 per cent of pre-epidemic stock;

(3) that survivors were wholly or partly resistant and bred similar progeny;

(4) that the progeny became fully resistant (i.e., less than 10 per cent per year died) in about thirteen years from the first disease mortalities.

In the next twenty-five years this disease destroyed all the oyster fisheries of Prince Edward Island. Perhaps the most interesting outbreak was the one affecting Hillsborough Bay and it tributary rivers, commencing in 1935, because it was observed from its onset and because resistance was studied and attempts were made at rehabilitation. Observations disclosed a spreading epidemic of much the same nature as in Malpeque Bay, except that mortalities reached or exceeded 95 per cent. In Johnston River, a small tributary, a relatively large number of resistant Malpeque adults were planted to act as brood stock in assisting rehabilitation. This had no apparent effect, the development of full resistance taking thirteen years, as in Malpeque Bay. In a further effort at rehabilitation, resistant Malpeque Bay spat were sold, in comparatively small numbers, to growers in Hillsborough Bay and the principal tributaries. The fishery recovered to its pre-epidemic level in ten to twelve years, as compared to the twenty years required in Malpeque Bay. It is uncertain whether this was accomplished by direct survival of these spat to market size or by their spawning of resistant young or by a combination of these two.

The causative organism of these epidemics was not discovered.

Pertinent papers are those of Needler (1931, 1936 and 1941), Homans (1937, 1938 and 1939), Fraser (1938), Richardson (1938), Needler and Logie (1947) and Logie (1958). The last two are the most comprehensive.

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FRB No. 678

From 1947-50 and again from 1953-55, the senior author studied model or miniature epidemics in small lots of mainland oysters placed in the waters of Bideford River, tributary to Malepeque Bay. From these experiments it appeared:

(1) that the causative organism was still present in Malpeque Bay waters;

(2) that mainland oysters were susceptible;

(3) that, with few exceptions, mortalities followed a characteristic pattern of a one-year lag, presumably for development of the disease in the oyster, followed by heavy mortality reaching 95 per cent of the original number in three years and a probable terminal value of 99 per cent; the heaviest rate of mortality occurred in the third year of exposure and reached 75-80 per cent.

It is probable that all Prince Edward Island epidemics conformed closely to this description. The apparently increasing mortality in successive outbreaks was more probably the result of closer observation than of increasing death rate.

In 1955, oysters commenced dying in mass numbers in various waters of New Brunswick and Nova Scotia. This epidemic is still advancing, the sole area of any importance not yet clearly involved being Bras d'Or Lake, N. S. Mortalities follow the same pattern and reach the same intensity as described for Prince Edward Island.

The normal oyster fishery of the mainland is relatively large, that of New Brunswick in particular being two to three times that of Prince Edward Island. Rehabilitation of the fishery in these provinces was therefore a pressing problem. The urgency was increased by another consideration. Returns from the oyster fishery provided in many cases an essential segment of the income of people in a chronically precarious financial state. Rapid restoration of the fishery was therefore an urgent socio-economic measure.

Even considering its poor success in Johnston River, the most promising technique at the time was the transfer of resistant Prince Edward Island adults to disease-depleted mainland waters to act as brood stock. In orderly scientific procedure it would have been necessary to discover the answers to the following questions before massive transfers took place:

(1) Was this the same disease as in Prince Edward Island?

(2) Would Prince Edward Island adults survive in the mainland waters?

(3) Would they spawn?

(4) Would their progeny be resistant?

However, because of the urgency of the socio-economic problem, preliminary search for these answers and large-scale rehabilitation transfers went on almost concurrently.

MATERIALS AND METHODS

Commencing in 1956 and continuing every year thereafter, two lots of 300 Prince Edward Island oysters were held in trays in several locations in mainland waters. Mortalities were regularly observed in these and in comparable lots of native and of Bras d'Or Lake oysters held as controls.

Commencing in 1957 and continuing into 1960, large numbers of adult Prince Edward Island oysters were transplanted to the principal diseased areas of the mainland to serve as brood stock. In all, some 4 to 5 million oysters were so transplanted.

Commencing in 1957, mortalities in tray lots of spat annually produced in diseased areas were observed. Since 1958 two controls have been employed:

(1) Malpeque Bay spat (presumably resistant);

(2) Bras d'Or Lake spat (presumably susceptible).

In addition general observations have been made of the numbers of spat and small oysters appearing on natural and artificial cultch in the diseased areas.

RESULTS

As already stated, the pattern of mortalities in mainland epidemics conformed in virtually every case and every particular with that established as characteristic of epidemic disease in Prince Edward Island.

The introduced oysters did live and did exhibit the same degree of resistance as in Prince Edward Island.

They did spawn. It should be stated that 1958, 1959 and 1960 were good spawning years and that 1959 was phenomenally so.

Large numbers of spat and of year-old oysters appeared in the diseased areas, but spat caught on artificial cultch and held on trays in these areas has died heavily, showing no clear disease resistance whatever. Similarly spat and small oysters picked up from around the shores died when held on trays.

DISCUSSION

The identity of mainland and of Prince Edward Island oyster diseases cannot be established by reference to the causative organism, since this has not yet been clearly identified. However, adherence to the characteristic mortality pattern everywhere and the similar resistance of Prince Edward Island oysters in Prince Edward Island and in mainland waters are together taken as the best available evidence that all epidemics are due to the same cause. It should be pointed out though that this is an inference from the data rather than a definitive statement (Logie, 1958).

The lack of resistance in spat produced in mainland waters after transfer of Prince Edward Island brood stock is disconcerting to the whole concept of rehabilitation by this method, although it has been pointed out by Drinnan and Henderson (1959) and by Drinnan (1960) that it conforms to the precedent established in Johnston River in 1939 et seq.

No completely satisfactory explanation of this phenomenon can be advanced, but Drinnan (1960) has speculated about it. Spat produced locally on the mainland now could be a mixture of three crosses:

(1) native x native;

(2) native x P.E.I. transplants;

(3) P.E.I. transplant x P.E.I. transplant.

Good reasons exist for suggesting that the first two of these should be highly susceptible and that the third should be highly resistant. Because of the paucity of surviving native adults demonstrated by searches so far, it was considered that progeny produced after rehabilitation transfers would be dominated by the third parentage listed above. In view of the data now at hand, it appears probable that somewhere in the diseased areas large enough stocks of surviving natives exist to exert a dominant influence on the genetic composition of the spat. This thesis is barely tenable in some cases, but it is nevertheless the only one consistent with the facts. Its authenticity should be tested by:

(1) searching for the postulated stocks of native parents;

(2) destroying them when found;

(3) saturating further with P.E.I. parents;

(4) continuing trials of spat survival, both in trays and on natural cultch.

In the meantime, judgment of this method of rehabilitation should be withheld.

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Summaries of Research 1959

A. Summaries by Countries

I. Canadian Research Report, 1959

A. SUBAREAS 2 AND 3

BY W. TEMPLEMAN

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Biological Station, St. John's, Nfld.

The Fisheries Research Board of Canada has carried out researches during 1959 in Subareas 2 and 3 on cod, haddock, redfish and American plaice. Hydrographic sections were taken in July-August over the area from southern Labrador to the southern Grand Bank.

Cod, *Gadus morhua* L. Otolith age readings for cod collected in Labrador, 1950-58, indicated the most successful year-classes to be 1942 and 1947, followed by moderately successful year-classes in 1944, 1945, 1946 and 1948 and very poor survival of the 1943 year-class. In collections of cod both inshore and offshore in Labrador, 1959, the 1950 and 1952 year-classes were most abundant and the 1949 year-class poorly represented.

A survey (intended to be repeated annually) of baby cod in the inshore areas of Newfoundland was begun by seining on the beaches of the east coast. Cod of the 0+ group were most numerous, cod of the 1+ group relatively scarce and although only a few of the 2+ group were caught the fairly large number of this year-class present in the harbours is indicative of high survival from the 1957 spawning.

Observations on the inshore and the long-line fisheries were continued at Bonavista. This area has apparently been affected detrimentally by the combined effects of the local catch and of the large catch in the local and neighbouring areas in recent years by European trawlers and longliners. Although the cod landings in the Newfoundland inshore area were 40% higher in 1959 than in 1958, the increase at Bonavista was only 11% higher and only 9.6 million pounds were caught compared with 15.3 million pounds in 1957. Of the 1959 total, 45% was from handlines (both jiggers and baited hooks), 30% from traps, 5% from line trawls operated by hand from small boats and 20% from longlines.

In the period 1952-58 the inshore fishery at Bonavista was mainly based on a group of fish which in the handline catches had a modal length of 52-53 cm in 1952 and which gradually increased in size to a mode of 62-63 cm in 1958. This group has now been very much reduced and the future success of this fishery will depend on the strength of a new group of small cod which made their first appearance in the catch in 1959.

The cod-trap fishery in the St. John's area has been studied since 1957. In 1957 and 1959 trapping began in the latter half of June and continued to the middle of August or later. In 1958 cod trapping began early in June and ceased before the end of July. The winters of 1957 and 1959 were cold and fishing was somewhat delayed, but in both years cod were available to the traps (which are set in 8-20 fathoms) because of being concentrated inshore in the trapping areas by the cold, below 0°C, water layer underneath a shallow layer of warmer surface water. In 1958, on the other hand, the warm surface water extended deeply enough for cod to descend below the range of the traps by the end of July.

In the cruise of the *A. T. Cameron* to Baffin Island, Ungava Bay and northern Labrador from August 18 - September 14 (Fig. 1) no cod were caught north of Line V at Lat. 63°N where 2 individuals (19 lb) and 14 individuals (83 lb) were caught at 125 fathoms (-0.25°C) and 150-160 fathoms (1.13°C) respectively. A few cod were caught off Frobisher Bay in Lat. 62°14′ to 62°20′N in 150-200 fathoms. In Ungava Bay cod occurred in small numbers (3-64 indididuals, 20-215 lb) in all 7 half-hour hauls in 27-200 fathoms (-0.6 to 2.0°C). No commercial concentrations of cod were encountered on the cruise, the greatest catch being 500 pounds in a half-hour's dragging at 99 fathoms (-0.4°C) off Cape Mugford, Labrador, Lat. 57°33′N. On the other

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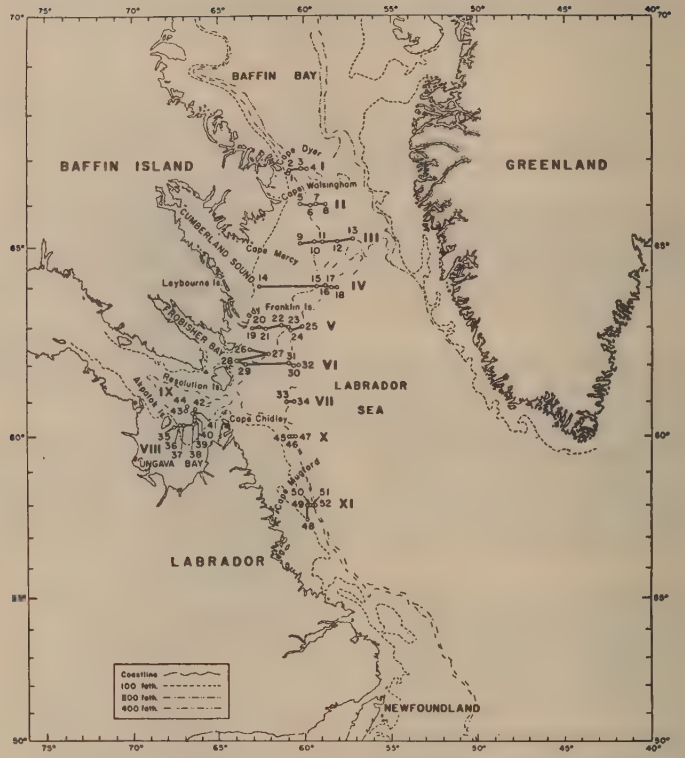

Fig. 1. Cruise of R.V. "A. T. Cameron" to Baffin Island, Ungava Bay and Northern Labrador, August 18 - September 14, 1959. Position of trawling stations.

hand ice conditions and the necessity to investigate the deepwater redfish areas limited the amount of shallow-water exploration on this cruise.

Haddock, *Melanogrammus aeglefinus* (L.). The annual otter-trawl survey for haddock was made by the *Investigator II* on the southern half of the Grand Bank from May 1-9 in depths from 25-100 fathoms. At the same time the *A. T. Cameron* explored the deeper areas of the southwestern slope between 80 and 400 fathoms in a redfish-haddock survey. The survey of St. Pierre Bank was carried out by the *Investigator II* in June. In the intensive commercial haddock fishing by Newfoundland trawlers on the southwestern slope of the Grand Bank in late winter and early spring preceding the above survey, haddock had been concentrated much more and much deeper than usual. Catches as high as 25,000 pounds were often made in 15-20 minutes dragging. The haddock, also, were driven much deeper than usual, presumably by low temperatures due to the unusually cold winter. Whereas

usually in late-winter, early-spring most commercial fishing for haddock on the Grand Bank extends down only to about 80 fathoms, in March 1959 large catches of haddock were being made as deeply as 120-140 fathoms. By the time of the *Investigator II* and *A. T. Cameron* surveys these late-winter, early-spring pre-spawning concentrations of haddock had largely dispersed northward, probably to some degree at least pelagically, and the only moderately good catches obtained per half-hour's dragging were one of 5,000 pounds at 50 fathoms and two of 2,300 and 4,000 pounds at 125 fathoms on the southwestern slope of the bank. On St. Pierre Bank the 4 largest haddock catches per half-hour's dragging ranged from 360-900 pounds in 80-110 fathoms on the southwestern slope of the bank.

On the Grand Bank the most abundant group present was the 1955 year-class, with a mode in the length distribution at 33 cm, which accounted for nearly 50% by number of the research vessel catch. The once very abundant 1949 year-class had become reduced significantly in numbers and accounted for only 7% of the catch, whereas the moderately abundant year-classes of 1952 and 1953 together comprised 28% of the catch. The 1956 year-class which showed some promise in 1958 did not survive well and initially was probably not more than one-fifth as abundant as that of 1955. The 1957 brood appears to have been a complete failure and the same is true of the 1958 year-class.

From a survey of the deep-water areas along the southwestern slope of the Grand Bank, the 1955 year-class was abundant at all depth ranges from 47-50 fathoms to 117-196 fathoms, but there was a definite trend toward an increase in the number of haddock of the older year-classes with increasing depth. A cruise in July to the shallowest part of the bank revealed very large concentrations of haddock of the 1955 and 1956 year-classes in capelin-spawning areas at 23 fathoms on the Southeast Shoal of the Grand Bank, year-classes older than that of 1955 being almost completely absent from the catches. It would appear that pelagic or other movement of haddock from the deeper wintering areas to the shallower water of the Southeast Shoal of the Grand Bank in 1959 was limited mainly to the younger year-classes, whereas the older mature haddock remained in the deeper water along the slope and did not migrate so extensively. This is similar to the habits of cod off northeastern Newfoundland—the large older fish remaining all summer in the deep water and the younger fish moving pelagically toward the shallower water which in this area is only to be found inshore.

The length frequencies and the age composition of the commercial landings of haddock from 1957-59 show the rapid reduction in abundance of fish of the once very abundant 1949 year-class. The 1952 and 1953 year-classes were moderately successful and contributed significantly to the catches during 1957, 1958 and the early part of 1959, but they are declining in abundance rapidly. At present, the 1955 year-class is the only very abundant one on the Grand Bank and haddock of this year-class are only now becoming of marketable size. There is no evidence of any significant survival of year-classes since that of 1955, and even if the 1959 brood has been successful in surviving, the 1955 brood alone cannot be expected to maintain the haddock fishery at a high level for the 5 years required for haddock of the 1959 year-class to grow to marketable size.

Large quantities of small Grand Bank haddock (30-40 cm) appeared inshore along the eastern coast of the Avalon Peninsula during late June and July 1959 and large numbers were caught by cod traps. They appeared to move northward along the east coast and did not remain in one place for more than a few days. The appearance of haddock in quantity along the east coast is unusual, and may have been due to cold Labrador Current water, unsuitable for haddock, covering the bottom of the Grand Bank in early summer, thus forcing the haddock to assume a pelagic existence in the warmer water near the surface. (Bottom water temperatures on the southwestern Grand Bank in the summer of 1959 were lower than usual and relatively unsuitable for haddock.) In the pelagic phase, in the rather shallow surface layer of warm water over water too cold for the haddock to go to the bottom, the vertical movements are restricted and the horizontal movements greatly increased

until finally the haddock reach the only shallow water available near the western Grand Bank region, i.e. the water near the coast of the Avalon Peninsula. On the eastern side of the Grand Bank there were during the summer months correspondingly great concentrations of haddock of the same size group (30-40 cm) in the shallow water of the Southeast Shoal.

In October and November haddock were caught by otter trawlers on shallow banks in the vicinity of Cape Race and catches of up to 5,000 pounds per set were obtained. These probably were part of the schools which migrated northward earlier in the summer and by late autumn had moved southward again.

There has been very little commercial fishing for haddock by Newfoundland trawlers on St. Pierre Bank since the winter and early spring of 1956. It appears unlikely that there will be a significant fishery for haddock there in the near future, as there has been a very low survival of all year-classes since that of 1949.

Redfish, *Sebastes marinus mentella* Travin and *Sebastes marinus marinus* (L.). A study, based on otolith age readings, of the age and growth of *mentella*-type redfish in Hermitage Bay, indicates that at the smallest length at which redfish are acceptable in the Canadian fishery, 25 cm, male redfish are about 10 years old and female redfish approximately 9 years old. At a length of 30 cm males are about 16 years and females 12 years of age. At 35-38 cm which represents the usual peak sizes of the larger redfish from the Gulf of St. Lawrence and from the southwest coast of Newfoundland, male redfish are on the average more than 30 and females 19-25 years of age. Some specimens of both sexes apparently live to 50 years of age or more, but in Hermitage Bay only about 2 or 3 centimetres additional growth in length occurs between 24 and 48 years of age.

In 1959 the *A. T. Cameron* made 4 redfish cruises in Subareas 2 and 3. A No. 41 otter trawl was used on a series of lines usually in standard depths from 100-400 fathoms. Bottom temperatures and bathythermograph records were obtained at each fishing station and dragging was for a half-hour on bottom in each case. In a survey of the southwestern part of the Grand Bank in May-June between 80 and 400 fathoms only 2 good redfish catches were made, 5,800 pounds (averaging 1.3 lb) at 290 fathoms and 4.0° C in the mid-southwestern slope and 13,000 pounds of small redfish (average 0.7 lb) at 125 fathoms at the southern tip of the bank at Long. 50°31′W. All other catches were of 3,000 pounds or less. The *A. T. Cameron's* fishing with the larger No. 41 net on the southwestern slope of this bank was notably less successful than the fishing of the *Investigator II* with the smaller No. 36 net in the same area in May-June 1952 and especially in early May 1951.

On the southwestern Grand Bank almost all the redfish fishing is done by United States vessels and there appears to be a great reduction of the redfish concentrations found in earlier years. Redfish increased in size with increase in depth. Previous to the *A. T. Cameron* cruise to the southwestern Grand Bank, in which 26 *marinus*-type redfish were caught, there were only 8 definite records of *marinus*-type redfish from the southwestern slope of the Grand Bank. Most *marinus*-type redfish were caught at 125 fathoms and none occurred at depths greater than 200 fathoms.

Investigations on the eastern slope of the Grand Bank were carried out between June 10 and July 21. On the southeastern part of the bank 3 large catches (9,600, 7,100, 8,400 lb; average weights 0.6, 0.7 and 1.1 lb) were taken in 165, 150 and 195 fathoms respectively, at bottom temperatures between 2 and 3°C. Northward toward the middle and northeastern Grand Bank larger redfish were found, the largest catches being 6,000, 4,200 and 2,400 pounds at 170-175 fathoms (average weights 1.3, 1.5 and 1.0 lb, bottom temperatures between 2 and 3°C), and a catch of 4,200 pounds at 200 fathoms and 1,900 pounds at 250 fathoms (average weights 1.5 and 1.9 lb, bottom temperatures 3.0 and 3.4°C). No catches of commercial quantities of redfish were found below 250 fathoms, and at 400 fathoms, the greatest depth fished, the average catch of redfish in 6 drags was 3 fish.

Along the eastern slope of the Grand Bank, especially northward, commercial quantities of redfish lay deeper than on the southwestern slope

and on the southern tip of the bank. This was partly due to the presence of considerably larger redfish and the relative scarcity of small redfish in the northeastern area. With occasional exceptions, and these generally in small catches, the sizes of redfish increased with depth.

During this survey of the eastern slope of the Grand Bank 83 *marinus*-type redfish were caught, 70 of these in a single set at 125 fathoms, 2°C, at the southeastern corner of the bank. With the exception of one *marinus*-type fish taken at 300 fathoms all the *marinus*-type redfish occurred in depths of 150 fathoms and shallower. On each line of stations crossing the slope from shallower to deeper water the large *marinus*-type fish occurred at shallower depths than the peak catch of the smaller *mentella*-type fish.

Thirty successful sets were made on the northeastern Newfoundland Shelf from September 27 - October 5, at and near the continental slope area from Hawke Channel (off Hawke Harbour, Labrador) to northeast of Fogo Island. The two most northerly lines were in the area immediately south of Hawke Channel where a very great fishery for redfish was developed in 1958. In 1959, up to October, both commercial and research vessel catches of redfish in this whole area proved to be disappointing. Of 30 successful sets by the *A. T. Cameron* only 4 (1,700-2,600 lb per half-hour's dragging) approached minimum commercial quantity. One of these catches was taken at 135 fathoms (average weight 2.1 lb), 2 at 250 fathoms (average weights 1.7 and 2.1 lb) and the largest catch at 300 fathoms (average weight 2.3 lb). All 4 of these catches were obtained at bottom temperatures above 3°C. The sizes given above are of *mentella*-type redfish which usually made up almost all the catch. These *mentella*-type redfish were much larger than those of the southwestern and southeastern Grand Bank and somewhat larger than those of the northeastern Grand Bank. *Mentella*-type redfish in this area were situated more deeply than in the areas to the south. The average catch in 4 sets at 400 fathoms, the greatest depth fished, was 26 redfish (average weight 2.1 lb).

Although the *marinus*-type redfish were far outnumbered by the *mentella* type they were considerably more abundant than in the more southern areas and over 300 *marinus*-type redfish weighing 1,300 pounds and averaging 4 pounds were caught in 30 sets. The *marinus*-type fish were as usual much larger than the *mentella* type. They also lay considerably shallower, being most numerous at 140-200 fathoms. A total of only 12 *marinus*-type redfish occurred in 8 sets at 250 and 300 fathoms and none below 300 fathoms.

The *A. T. Cameron* cruise of August 18-September 14 to Baffin Island, Ungava Bay and northern Labrador was to an area which, although largely north of Subarea 2, provided information of importance for understanding the redfish of Subarea 2. Otter-trawling stations on this cruise are shown in Figure 1. Beginning with a line of stations off Cape Dyer at the southern end of Baffin Bay in Lat. 66°42′ to 66°48′N and continuing in a group of stations, usually on each degree of latitude line as far south as Lat. 58°N off Labrador and also in Ungava Bay, fishing by otter trawl was carried out in depths generally of either 100 or 150 fathoms or both and 200, 250, 300 and 400 fathoms.

On Line I off Cape Dyer only 2 small redfish were found and on Line II, Lat. 66°N, only one. One hundred and fourteen redfish were obtained on Line III at Lat. 65°N. Only a few small redfish were found in Ungava Bay. No commercial catches of redfish were obtained over the whole area. The greatest quantities in a half-hour's dragging were 500 pounds at 297 fathoms (2.3°C) on Line IV at Lat. 64°N and 800 pounds at 305 fathoms (3.8°C) on Line VI at Lat. 62°N.

Apart from a total of 11 large *marinus*-type fish obtained (10 at 300 fathoms and 1 at 400 fathoms) on all lines from Lat. 65° to 62°N all redfish were definite *mentella*-type fish of the Labrador-northern Grand Bank type. Although many large females were present all female redfish were immature southwards to Lat. 62°N. Small 6-13 cm redfish were present from Line IV off Cumberland Sound (Lat. 64°N) to Ungava Bay and were especially plentiful (173 individuals) at 155 fathoms (1.1°C) on Line V north of Frobisher Bay (Lat. 63°N) but none were found on the

two lines at Lat. 60° and 58°N, off northern Labrador.

The northerly distribution of the young redfish over areas where the Labrador Current was proceeding rapidly southward and where all the female redfish were immature, strongly indicates that these young redfish are the result of a drift of redfish larvae from West Greenland.

American plaice, *Hippoglossoides platessoides* (Fabr.). From the 1,000 American plaice tagged on the northeastern slope of the Grand Bank in 1954 approximately 5% have been recaptured. Most of these were taken within a 75-mile radius of the tagging locality. However, a few returns indicated a general westward and southwestward movement; 1 tagged fish was recaptured near Sable Island. The St. Mary's Bay tagging of 1954 seems to indicate no movement of plaice from this coastal bay to offshore bank areas. One thousand plaice were tagged on the southern and southeastern slopes of the Grand Bank during the past year.

A study of the catch of plaice per unit of effort by commercial trawlers on the Grand Bank for 1953-58 indicates a sustaining yield in the northern and northeastern part of the bank, a fluctuating condition on the central eastern slope and a somewhat declining trend on the southern and southeastern slopes.

Hydrography. The usual six hydrographic sections across the Labrador Current, the banks and continental shelf from Labrador to the southern Grand Bank were taken from July 25-August 26 by the *Investigator II*.

The winter of 1958-59 was colder than usual in eastern Newfoundland whereas the winter of 1957-58 was relatively warm. The mean air temperatures at Torbay Airport, near St. John's, in December-March were 23.3, 30.9 and 21.8°F (-4.8, -0.6, -5.8°C) in 1956-57, 1957-58 and 1958-59 respectively, compared with the long-term average of 26.5°F (-3.1°C).

In the Seal Islands section (Fig. 2), off Labrador, temperatures of the cold intermediate layer were little different from those of 1958. The volumes of water with temperatures below 0°C were quite similar in the 2 years, possibly a little less in 1959, but water with temperatures below 0°C and below -1.0°C in the inshore area was much closer to the surface in 1959 than in 1958. The deep water to the east of Hamilton Inlet Bank in 1959 was slightly warmer than in 1958 but not nearly as warm as in 1957 when the deep-water temperatures were unusually high. Water with a temperature as low as -1.5°C was not noted in 1959 and existed at only one level at one station in 1958.

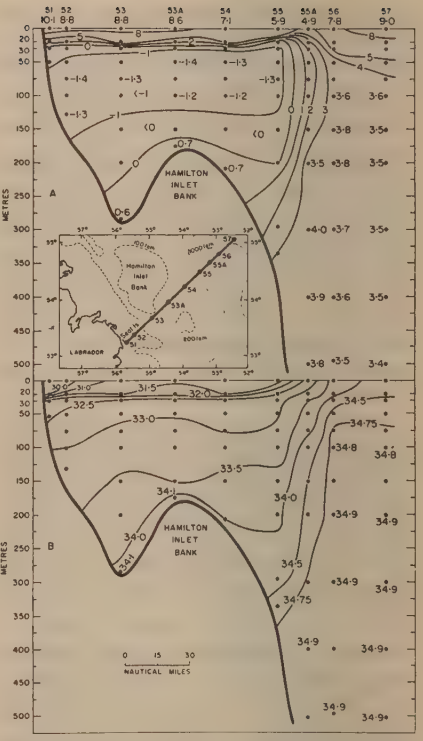

Fig. 2. Hydrographic section off Seal Islands, across Hamilton Inlet Bank, Labrador, August 7-10, 1959. A—Temperature °C; B—Salinity per mille.

In the St. John's-Grand Bank-Flemish Cap section (Fig. 3) the general temperature system

at all depths resembled that of 1957. (The winter of 1956-57 was unusually cold in eastern Newfoundland but not as cold as that of 1958-59.) There was considerably more water with temperatures below 0°C and below -1°C than in 1958, but, as also occurred in the section off Bonavista, temperatures at the surface and in the water layer shallower than 25 metres were higher in 1959 than in 1958.

On the whole throughout the six sections, temperatures in the northern sections resembled those of 1958, in spite of the much colder winter (at least in eastern Newfoundland) in 1958-59 than in 1957-58. Progressively southwards temperatures were lower in 1959 than in 1958, and on the southwestern slope of the Grand Bank the temperatures of the deeper water in 1959 were considerably lower than usual.

In the summer of 1959 the Atlantic Oceanographic Group at St. Andrew's, N. B., completed the fifth of a series of seasonal surveys covering the Atlantic waters off Newfoundland. Variations in the Labrador Current and the slope water give rise to extreme variations of temperature and salinity over the banks. In March, for example, the temperature distribution over the Grand Banks suggests an exceptionally large flow of Labrador Current water and abnormal local cooling in 1959, as compared with 1958. A study of variations in the thermal structure of the Labrador Current indicates that this water moves south in bodies which at times seem to be semi-independent.

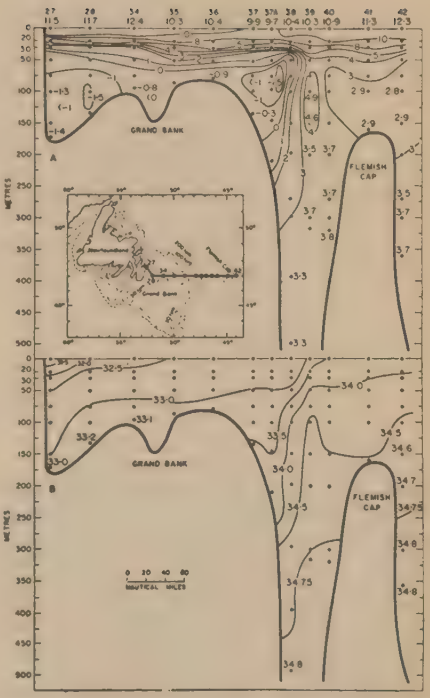

Fig. 3. **Hydrographic section St. John's - Grand Bank - Flemish Cap, July 25-27, 1959. A—Temperature °C; B—Salinity per mille**

I. Canadian Research Report, 1959

B. SUBAREAS 4 AND 5

By W. R. MARTIN

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Canadian research pertinent to ICNAF is conducted by the Fisheries Research Board of Canada and the Quebec Department of Fisheries. The Board's research on commercial fisheries in Subareas 4 and 5 is largely the responsibility of the Biological Station at St. Andrew's, N. B. Hydrographic research is done by the Atlantic Oceanographic Group of Halifax, N. S. These programs are providing predictions of fishing success under various natural conditions and with conservation measures.

Groundfish field work included studies of commercial operations and investigations from research vessels. The main species studied were cod, *Gadus morhua* L., haddock, *Melanogrammus aeglefinus* (L.) and American plaice, *Hippoglossoides platessoides* (Fabr.). Commercial fishing was assessed by a statistics and sampling program on landings at fishing ports (reported elsewhere), and by making sea trips on commercial trawlers to sample the quantities and sizes of groundfish discarded at sea. Research

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vessels were used to examine fish distribution, movements and growth in relation to the environment, and to define the selective properties of fishing gears. The major development in 1959 was use of the new offshore research vessel *A. T. Cameron*, which permitted extended coverage of offshore banks during the winter season. IBM methods are being introduced to improve efficiency in handling voluminous statistics and research vessel data.

Discards at sea. Observations on discards of groundfish from otter trawlers were made in two areas, Nova Scotian Banks and the western Gulf of St. Lawrence.

On Nova Scotian Banks estimates of sizes, numbers and weights of haddock discarded during the months of May to August are available for two periods. The first period, 1951-53, was prior to mesh regulation when nets were about $2\frac{7}{8}$ inches internal mesh. In the second period, 1958-59, all vessels were using meshes of 4 inches or more, but with various sizes and kinds of top chafing gear. Size compositions of haddock catches and landings for sea sampling trips are presented for the years 1952, 1953, 1958 and 1959 in Figure 1. The numbers of haddock measured at sea per year varied from 3,000 to 8,000 fish. Percentage discards are summarized as follows:

Year	Condition of fish landed	Discards % by Weight	Discards % by number
1952	gutted	31	62
1953	gutted	20	44
1958	round	2	5
,,	gutted	10	22
1959	round	8	16
,,	gutted	17	35

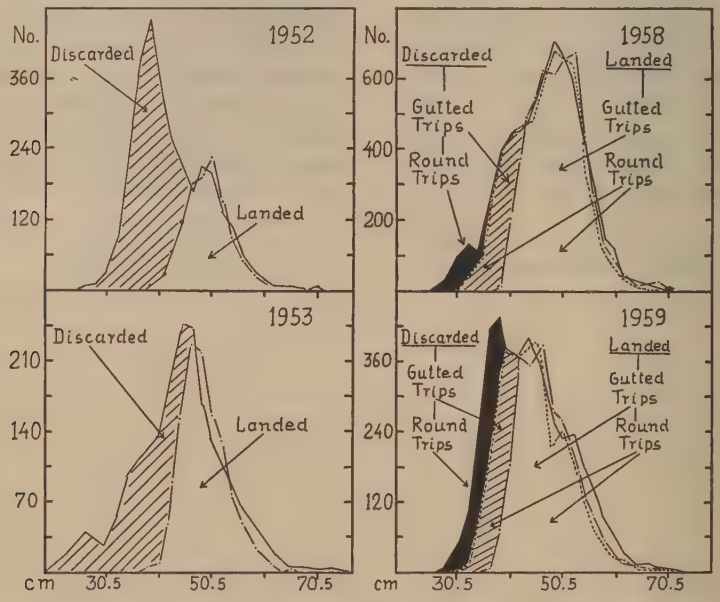

Fig. 1. **Size compositions of haddock caught and landed in 1952-53 and 1958-59, Nova Scotian Banks, showing sizes and estimated proportions discarded and landed.**

Lower discards in the later period are related to the larger mesh size and the lower minimum size of marketable fish. Annual changes in the numbers of small haddock available to the fishery also caused variations in the quantities discarded.

Observations of low cod discards on Nova Scotian Banks in 1958 and 1959 agree with earlier observations. For trips sampled in 1958-59 cod discards were about 10 to 15% by number and 4 to 5% by weight. The mean size of discarded cod was about 38 cm (¾ lb) and all cod larger than about 48 cm (1¾ lb) were retained.

Estimates of proportions of incidentally caught species discarded at sea were made in 1958 and 1959. Large proportions of hake (*Urophycis*), yellowtail (*Limanda*), skate (*Raja*) and silver hake (*Merluccius*) were discarded as unmarketable fish. However, only in the case of hake did discarding seem to be on the basis of fish size.

Proportions of groundfish discarded at sea were observed on nine sea trips in the western Gulf of St. Lawrence. Observations were from late May to early September on draggers using 4½-inch mesh codends. Cod discards ranged from 1 to 22% by number and from 0.4 to 11% by weight. Early in the season average discards were comparatively low at 3% by number and 1% by weight. During three later trips discards were 22% by number and 11% by weight. The change reflects an increase in the minimum size of fish acceptable at landing ports, and consequently at sea.

Wastage of American plaice in the same area amounted to 85% by number on two trips. This reflects the large numbers of fish between the 50% selection point (23 cm) for 4½-inch manila meshes and the 50% cull point (34 cm) for commercially acceptable sizes. Survival of discarded plaice returned to the water is being investigated.

Witch flounders (*Glyptocephalus*) were all large in size and none were discarded. White hake discards were 95% by number, with only the largest fish retained for markets.

Tagging. Tagged groundfish provide useful information on definition of stocks, migrations, growth and mortalities. Numbers of tagged haddock released in 1959 were 522 on Emerald Bank in March, and 218 on Sable Island Bank in September. Numbers of cod tagged were 469 on Banquereau in February, 65 on Emerald Bank in March, 1,398 in the western Gulf of St. Lawrence (4T) in August-September, and 112 on Sable Island Bank in September. Tags were attached to 879 American plaice in the southwestern Gulf of St. Lawrence. Most of the tags were Petersen yellow plastic discs, attached by a stainless steel wire through the back of the fish.

Low recoveries from offshore taggings of haddock and cod probably reflect high tagging mortalities and possibly low fishing mortalities. Recoveries of Gulf of St. Lawrence plaice were highest at 37% per year in the Shippegan Gully area where there is concentrated fishing in May and June.

Returns of cod and haddock tags support earlier observations of seasonal migrations. No definite migratory patterns have been observed for plaice, although a few individuals moved distances up to 100 miles.

Surveys. Research vessel surveys on Nova Scotian Banks and in the southwestern Gulf of St. Lawrence were continued in 1959. These surveys provide assessments of the effects of environmental factors on abundance and distribution of fish. They also give a basis for predicting recruitment of young fish before they enter the fishery. On the Nova Scotian Banks emphasis was on haddock, and in the Gulf of St. Lawrence on cod and plaice.

In summer small haddock under 40 cm were taken most abundantly in shallow water (less than 30 fathoms) on top of the banks. In winter they had disappeared from this shallow water, but large catches were taken from deeper water along the edge of the banks, between 60 and 120 fathoms.

Large haddock were not numerous anywhere in summer, except for a very small area on top of Emerald Bank. In winter large catches were made at 50 to 60 fathoms around Emerald Bank.

In summer small haddock were most abundant in warm shallower water above 7°C. In

most areas temperatures do not seem to have been limiting, but temperatures between 1° and 3°C over much of Banquereau probably reduced abundance there.

During winter haddock were absent from the shallow water where temperatures less than 1°C prevailed. Between 1° and 3°C small haddock predominated in the catches, and their numbers increased in the warmer portion of this temperature range. Large haddock were most abundant at bottom temperatures between 3° and 5°C, at depths of 50 to 60 fathoms, in the vicinity of Emerald Bank.

The 1956 and 1957 year-classes of haddock are relatively numerous. Modal lengths for these year-classes were about 30 and 20 cm in August 1958; 34 and 24 cm in March 1959; 34 to 36 and 28 to 30 cm in July 1959. The 1956 brood is expected to start contributing to the commercial fishery in 1960, and the 1957 brood in 1961. This should result in above-average abundance of haddock in 1961 and 1962. There are indications, however, that the 1958 year-class is weak and a return to normal catches is predicted for about 1964.

In 1959 the Gulf of St. Lawrence cod survey was continued for the third consecutive year and was extended to the Cape Breton coast, along the western edge of the Laurentian Channel.

Distribution of cod throughout the survey area varied with fish size. Small fish, 31 cm and less in length, were more numerous in the shallower Shippegan Gully area where temperatures were higher in summer and early fall. Larger cod were more abundant in cooler waters of the northern part of Subdivision 4T.

The size composition of cod caught off the Cape Breton coast in May is similar to that found near the Gaspé coast in June. The size composition of smaller catches of cod off Cape Breton in November is similar to that for Gaspé area cod in October. The interpretation that cod caught south of the Gaspé coast in summer months are found off Cape Breton in winter agrees with results of earlier tagging experiments.

Analysis of catches in relation to bottom temperatures showed that in summer in the western Gulf of St. Lawrence highest catches of cod are found between -1° and 5°C. This is a wider range than the 0° to 4°C found in spring off Cape Breton, or the 1° to 3°C preferred temperature range in winter on Nova Scotian Banks.

The success of longline fishing for cod south of the Gaspé coast has been studied in relation to seasonal and annual variations in the distribution of cod food and temperatures. When euphausiids were abundant in plankton samples, they were also abundant in cod stomachs. Longlines set on bottom and floated at 10 and 20 fathoms from the bottom showed vertical migrations of 51 to 70 cm cod in August. These movements were related to the vertical distribution of euphausiids and the sharpness of the thermocline.

The main features of the dragger cod fishery in Subdivision 4T may be predicted from survey catches. On the basis of the 1958 survey it was predicted that the 1954 year-class would replace the 1953 year-class and that these small cod would be dominant in the 1959 landings (refer Canadian Research Report, 1958). Five-year-old cod of the 1954 year-class were in fact dominant in the 1959 landings. These cod had a mean length of 50 cm.

Survey catches of 1959 indicate that the 1955 year-class was the dominant one in the 3rd quarter of the year. The mean size of these 4-year-olds was 40 cm. It is expected that the 1955 year-class will be the dominant one in 1960 landings. No increase in the small average sizes landed is expected. In fact, average sizes of cod landed in 1960 may be smaller than those landed in 1959. Age determination of otoliths collected in 1959 indicates that the mean lengths of nearly all year-classes were smaller in 1959 than in 1958. If no change in growth rate occurs, the 1955 year-class will have a mean length of about 45 cm at age 5, compared with 50 cm at age 5 for the 1954 year-class.

Cod of the 1956 year-class were taken in large numbers in 1959 survey catches. They were almost all released by the 4½-inch mesh codends used by commercial draggers. Larger numbers of the 1956 year-class will appear in

the commercial catches in 1960, but most will probably be discarded. They will not contribute significantly to landings until 1961.

Growth Studies. A special study has been made to relieve uncertainty in interpreting the first annual check in otoliths from cod taken in the western Gulf of St. Lawrence (4T). Collections of small cod were made by using small-mesh liners in shrimp trawl and No. 36 nets. Length compositions showed a peak of 6 cm for young of the year in October. The age I mode was 13 cm in June and 17 cm in October. The age II peak was at 19 to 20 cm in June. Examination of otoliths of these fish showed that the small central hyaline zone should be counted as an annual ring in making age determinations.

Even with satisfactory age determinations for individual cod, it is difficult to obtain suitable average growth rates because of variations among fish from different sources. Cod distribution changes with fish size and season, and the various fishing gears differ in their selective properties. Some of the difficultes have been explored for 4T cod: (1) There appears to have been a substantial increase in average size at age of 5- to 10-year-old fish between 1949-52 and 1955-58; (2) Cod appear to be segregated by size in their seasonal movements with resultant effects on growth curves; (3) Line-caught fish above 8 years of age are larger than otter-trawl caught cod of the same age. In predictions it is necessary to recognize the complexity of average population and fished-stock growth curves.

A study of growth of American plaice from Subdivision 4T has been started. Beyond the age of 5 years (25 cm) females grow faster than males. Differences in size at age are shown between shallow- and deep-water stocks, confirming tagging observations of little movement. Plaice enter the commercial fishery (landings) at age 7.

Gear Selection. Equitable enforcement of mesh regulations requires knowledge of equivalent selectivity of manila meshes and the new synthetic twines which are being introduced to the commercial trawlers. The selectivity of the increasingly popular terylene codends was tested on haddock, cod and plaice. Terylene was found to be similar to other synthetic twines in releasing larger fish than manila meshes. The following mesh sizes of single-strand terylene are approximately equivalent to 4½-inch manila: for haddock 3⅞ inches; for cod 3⅝ inches; for American plaice 3⅞ inches.

The selectivity of large meshes in a cod trap was tested in July on the north shore of the Gulf of St. Lawrence (4S). An auxiliary back of 3.0-inch meshes was used to catch released cod. The 5.2-inch back released 64% of the total catch. The 50% selection point was 57.5 cm, giving a selection factor of 4.4. Lower selection factors were obtained in previous experiments, and the average selection factor is now 4.1 for a cod trap with 5.2-inch meshes. At times many cod were gilled in the large meshes.

Comparative fishing. In November the research vessel *Harengus* fished a No. 36 otter trawl for two days alongside a commercial Danish seiner, the *Cape St. Mary*. The otter trawl had a 4½-inch mesh, double manila codend and the Danish seine had a 3½-inch mesh nylon parachute cord codend. The seiner used 900 fathoms of rope on each side of the seine. The *Harengus* made four 1¼-hour and five 1½-hour hauls. The seiner made 7 sets. All tows were in daytime. Average catch per tow in pounds of the important species was as follows:

	Danish Seine	Otter Trawl
American plaice	1,471	346
Witch	254	14
Cod	239	263
Hake	321	125

The Danish seine caught larger plaice and smaller cod and hake than the otter trawl.

Sea Scallop. Four sea trips were made to Georges Bank on commercial draggers to gather information for abundance and mortality studies. As in previous years, the boats are able to saturate the shucking power of the crew, and hence it is shucking power rather than abundance which continues to limit the catches.

Canadian crews continue to discard over half the catch since these discards are too small to

make shucking profitable. The 50% cull point is between 95 and 100 mm shell height.

A preliminary experiment tested the effect of using 4-inch rings as opposed to 3-inch rings in the drags. Trials were made on a commercial boat in October and November. Results indicate that the larger rings are slightly more efficient at catching market-size scallops. The major advantage, however, is the big reduction in the amount of trash and small scallops landed on deck. Less time is needed to cull the catch and more time can be devoted to shucking.

Hydrography. The main feature of surface temperature changes from 1958 to 1959 was a decrease at all stations, except in the Gulf of St. Lawrence. The temperature conditions in deeper water layers seem to be the result of long-term cooling and also of intense cooling during the winter of 1958-59. A very cold intermediate temperature layer was still persistent on the Scotian Shelf in October 1959 (Fig. 2). The cold-water layer in the Gulf of St. Lawrence was observed to cover a much larger area of the bottom than in previous years.

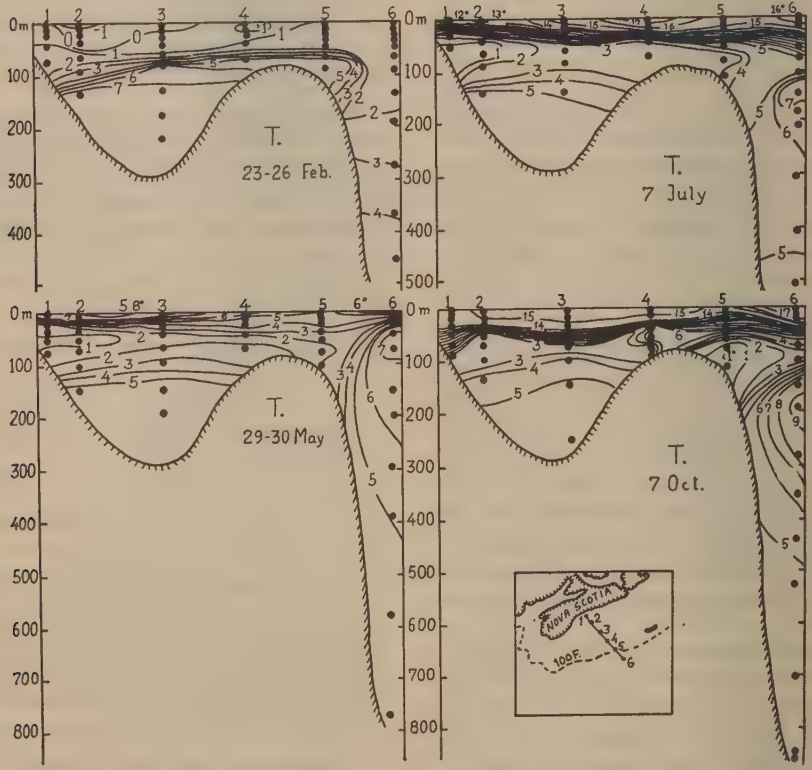

Fig. 2A. **Hydrographic sections off Halifax, 1959. Temperature °C.**

Analysis of temperature data from the deep waters of the Scotian Shelf and other areas has indicated a long-term cooling trend in the last decade from a maximum which occurred either in 1951 or 1953. There is evidence that some of the "cold" year conditions that existed during the thirties have been restored.

The cooling trend, first observed in the surface temperatures along the Atlantic Seaboard, is now known to be general for the deep waters at the entrance to the Bay of Fundy and on the Scotian Shelf. The St. Andrew's surface temperature variations have proved to be an excellent index for surface and deep water temperature variations in other areas.

The seasonal temperature variations of the bottom waters of the central sector of the Scotian Shelf have been studied. They indicate that the minimum temperatures occur during the second quarter of the year, and the maximum temperatures occur during the last quarter along the coast and during the first quarter on the offshore banks.

Drift bottle experiments have shown seasonal and year-to-year changes in surface circulation in the Bay of Fundy and the Gulf of Maine. In general, the results have tended to confirm the concept that the surface circulation pattern in the Bay of Fundy is "closed" in the winter and "open" in the summer.

A submarine geological program has been initiated in the Atlantic waters. Areas of investigation are centered around several major fishing banks in the Gulf of St. Lawrence and on the Scotian Shelf.

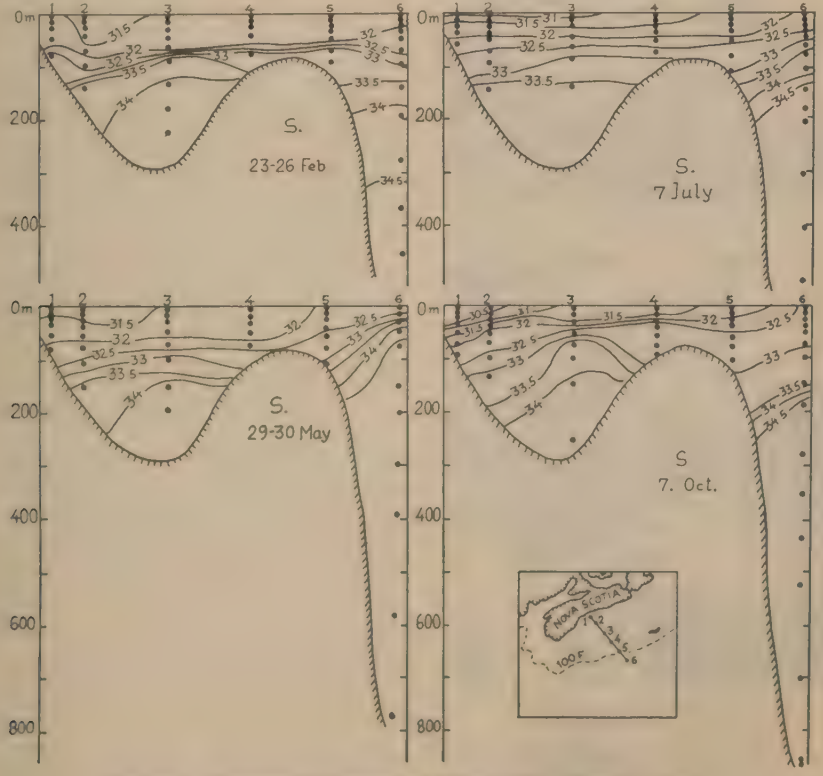

Fig. 2B. Hydrographic sections off Halifax, 1959. Salinity per mille.

Banding Lobster Claws--

A Better Way to Inactivate Them

By D.G. WILDER

WHEN LIVE LOBSTERS are crowded together during storage and shipment they often injure or kill each other. Such losses can be reduced if the lobsters are prevented from opening their claws. In Europe this is done by fastening twine, brass wire or rubber bands around the claws. In North America the most common method is to insert a small wooden or plastic plug in the thumb joint (Figure 1). A few fishermen cut the exposed thumb joint tendon so the lobsters cannot open their claws. Rubber bands are also used in a few areas.

Each of these methods has advantages and disadvantages. Plugging is effective, cheap and fast. In time, however, the meat around the plug turns dark. If the lobsters are stored for several months, the shell near the plug often erodes quite extensively. Studies in the State of Maine have shown that the wounds caused by plugging are almost always infected with a type of bacteria that kills lobsters when injected. This suggests that plugging may cause some mortality through infection. Some contend also that plugged lobsters are more likely to drop their claws. These difficulties can be overcome by using wide rubber bands. Some dealers have maintained that the bands slip off during storage. In addition, many have considered banding to be a slow, rather difficult operation and this has probably been our fishermen's chief objection to it. Recently, however, banding pliers have appeared on the market (Figure 2). With a little practice, lobsters can now be banded quickly and easily. Although tendon cutting is quick and easy, there was little information as to how effective it is in preventing losses.

To learn more about the effects of plugging, banding and tendon cutting during long-term storage, two experiments were carried out. In the first experiment 200 unplugged, two-clawed lobsters were obtained in March 1958 direct from fishermen. These market-sized lobsters, which averaged about a pound and a quarter in weight, were divided into four lots of 50. Both claws of one lot were plugged with machine-made basswood plugs. Another lot

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Figure 1 - Machine-made, wooden plug being inserted

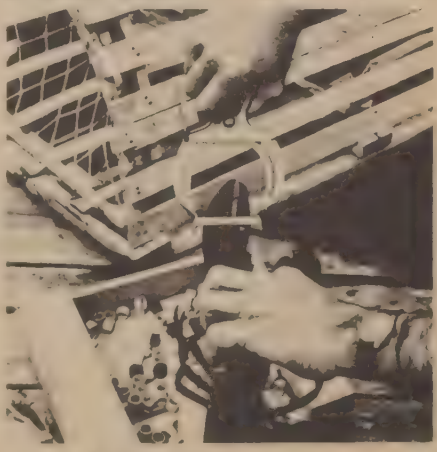

Figure 2 - Banding pliers in use

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was banded with half-inch wide rubber bands and the tendons of the third lot were cut with the point of a sharp knife. The claws of the fourth lot were not restricted in any way. Each lot of lobsters was checked daily and any deaths or loss of claws, plugs or bands were noted. Once a week all the lobsters were removed from the tanks and examined individually for injuries. All were fed regularly with salted herring. The experiment continued for four and one-half months.

Mortalities over the 4½-month period were as follows: plugged 54 per cent, banded 56 per cent, cut tendons 78 per cent, and untreated 98 per cent. It is clear from these results that lobsters survive better in captivity when their claws are inactivated. In this respect, plugging and banding do not differ appreciably. Tendon cutting, although better than no treatment, is not as effective as either plugging or banding. When the tendons were cut the lobsters bled quite freely for almost a minute, losing about half an ounce of blood on the average. This loss of blood did not, however, appear to be directly responsible for any of the deaths. From time to time, particularly during handling or when lobsters contacted each other, the tendon-cut claws opened. Although lobsters cannot open such claws deliberately, thay can close them normally. Injuries inflicted at such times undoubtedly caused some mortality.

During the experiment, the untreated lobsters lost 12 claws, the plugged lobsters two claws, the banded and tendon-cut lobsters none. It appears from this that loss of claws is not a serious problem if the lobsters are handled carefully.

Only one of the wide rubber bands came off and only two of the plugs came out during the 4½-month period. Apparently if suitable bands are properly applied, they remain in place as well as wooden plugs.

After the experiment was finished, 14 of the live plugged lobsters were boiled. In most of these the shell, meat and tendon near the plug were black. This blackened meat was judged to be definitely off flavour. In several, the shell near the plug was badly eroded.

INFECTION COMPARISONS

The second experiment was done to determine whether plugged lobsters are more susceptible to infection with Gaffkya homari, the bacteria that causes lobster blood disease. The experiment was carried on in the summer of 1960 during an epidemic of blood disease among lobsters stored near the mouth of the Bay of Fundy. One hundred and forty recently caught, unplugged lobsters were obtained from the southern Gulf of St. Lawrence where there was no evidence of blood disease. At St. Andrews, N.B., both claws of half the lobsters were plugged with hand-made, white pine plugs; the claws of the remainder were banded with rubber bands. The plugged and banded lobsters were stored together in a six-foot inside diameter, cylindrical tank. At the start of the experiment they were allowed to feed on two heavily-infected dead lobsters, and for the next 10 days, dead infected lobsters were suspended in the water. The experiment was continued for nine weeks.

The percentage dead in each lot and the percentage of those infected with blood disease at the end of the first and ninth weeks were as follows:

	Claws plugged		Claws banded	
	% dead	% of dead infected	% dead	% of dead infected
End of first week	59	97	16	100
End of ninth week	93	96	78	91

Plugged lobsters died faster and suffered higher mortalities than the banded. Apparently blood disease bacteria enter the body more easily when the surface layers have been broken by plugging.

To reduce losses during storage, lobsters' claws must be inactivated. Ordinarily, plugging and banding are equally effective in reducing losses and both seem better than tendon cutting. Plugging darkens the meat, erodes the shell and may produce off flavours. Plugged lobsters are more susceptible to lobster blood disease. For these reasons, banding is judged to be the better method of inactivating the claws.

MEASUREMENTS OF PRIMARY PRODUCTION IN COASTAL SEA WATER USING A LARGE-VOLUME PLASTIC SPHERE

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ABSTRACT

A free-floating 20-ft diameter thin transparent sphere, with its center 5.5 m below the sea surface, was filled with nutrient rich water filtered free from plants and animals and inoculated with a natural population of coastal phytoplankters. The resulting phytoplankton "bloom" was then studied in this well-mixed water mass under near-natural conditions of temperature and illumination with the minimum of complications arising from grazing, sinking or lateral transport of the plant cells.

Daily measurements were made of temperature, light and photosynthesis and the water was analyzed for oxygen, carbon dioxide and all common micronutrients. The production of plant material was followed by cell counts and pigment values and by analyses for particulate carbon, nitrogen, phosphorus, carbohydrate, protein and fat.

The results have a bearing on nearly all aspects of marine primary productivity and are discussed in detail. In particular, the growth kinetics and chemical composition of a natural mixed crop have been related to the environment with the minimum of ambiguity. Growth was finally limited by nitrogen deficiency, although the plant biomass nearly doubled after nitrate became depleted, with a shift of metabolism to carbohydrate and fat synthesis. The composition of the plankton at various stages in a bloom is summarized by ratios involving carbon and chlorophyll *a*.

Differences between the results for photosynthesis obtained by the oxygen and C^{14} method have emphasized the importance of knowing photosynthetic quotient values and possible unlabeled sources of plant carbon. During an initial period of low illumination there was strong evidence for heterotrophic growth.

The principal species found in the "bloom" were *Skeletonema costatum, Thalassiosira nordenskioldii, T. rotula, Gyrodinium fulvum, Glenodinium danicum, Nitzschia delicatessima* and *Asterionella japonica*. The last two grew very slowly and the two dinoflagellates only appeared after the nitrate was depleted.

INTRODUCTION

It is unnecessary to stress the difficulty of studying photosynthetic productivity in sea water that arises because of the impossibility of observing any one discrete body of water for a sufficient length of time. Valuable information can be obtained by observing the behavior of pure cultures of marine phytoplankton but such work is no adequate substitute for the study of the growth and decay processes that occur with mixed populations under natural conditions.

An obvious solution to the problem would be to impound sea water in a tank and valuable exploratory experiments of this nature were undertaken by Edmondson and Edmondson (1947) and Edmondson (1956). However, the use of a tank has several disadvantages unless it is very deep. The light-

Reprinted from Limnology and Oceanography, Vol. 6, No. 3, July, 1961, pp. 237–258.

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ing of the water mass as a whole can be unrealistically high, and undesirable temperature increases usually occur. Furthermore, no precise "balance" of dissolved oxygen or carbon dioxide can be readily computed when there is a large surface area of water in contact with the atmosphere.

The thin plastic tube experiment proposed by Margalef (1956) would avoid some of this trouble, but in order to reduce wall-effects (the undesired growth of epigenous organisms, especially bacteria) one must have as large a volume to surface area ratio as possible. It is now common experience that a wall-effect from bacteria can be noticed within a very few hours in a small bottle and we may therefore assume that a container must be many feet in diameter before wall-effects can be neglected for a period of many days. A large container has the additional advantage that errors from accidental contamination are reduced.

To overcome most of the difficulties outlined above we have constructed a free-floating thin plastic sphere, 20 ft in diameter, suspended with its center a constant distance (5.5 m) beneath the sea surface. This plastic bag was connected to the surface by a comparatively narrow neck through which samples could be taken. The state of the water contained in the sphere approximated closely that of the surrounding sea water, both in temperature and in the conditions of illumination. An account of the construction of this equipment has already been published by Strickland and Terhune (1961). It was used in Departure Bay (49°20′ N, 123°50′ W), near Nanaimo, on the east coast of Vancouver Island, British Columbia.

The purpose of the present paper is to describe a phytoplankton bloom which was observed in the plastic sphere under these near-natural conditions. The production of such a bloom has enabled growth conditions to be studied without any of the ambiguities normally introduced by water movement. Further, the detailed analysis of the resulting crop has given the first extensive set of results applicable to detritus-free marine phytoplankton grown under natural conditions, that has so far been reported. Phenomena such as the succession of species and the effect on phytoplankton of nutrient environment were studied with the minimum of complications.

In one experiment the plastic sphere was torn after only 5 days but in the other (June–July 1960) 22 days of growth and decay were recorded before the equipment commenced to leak and this experiment forms the basis of most of the results reported in the present communication. We propose to repeat the experiment at least once more, and expect to be able to prolong it for many more weeks so that the decay processes of an initial bloom can be studied in greater detail. There is really no end to the number of such experiments that could be undertaken as each time different species of plant cells would doubtless develop. The present work, however, measures the production of a diatom crop which is quite typical of the spring bloom in temperate coastal seas.

In this experiment some difficulty was encountered with the stirring mechanism and it was not possible to keep all dead and dying plant cells in uniform suspension within the bag. In these circumstances one cannot equate the formation of soluble metabolites to the decrease of particulate matter sampled at the center of the bag. However, this limitation applied only to the last few days of the decay processes which followed peak phytoplankton production.

The authors would like to record their thanks to L. D. B. Terhune and others at the Pacific Biological Station, Nanaimo, for their work in designing, assembling and launching the equipment. We also wish to acknowledge the assistance given by the undergraduate student assistants, Wing Wai and John Yu, in the analytical program.

PROCEDURE AND METHODS

General procedure

The plastic bag was filled with water taken near the sea floor, about 350 ft from the low-tide shore line. The depth of the water at the intake varied with the tide between some 15 and 20 m. The water was pumped to land, filtered through a cloth-plate filter coated with diatomaceous earth,

and returned to the location of the bag (250 ft out in 11–16 m of water) by an all-plastic pipe system. Volumes were recorded with a water meter and a total of 125 m^3 of sea water was required to fill the bag.

After preliminary analyses the contents of the bag were "inoculated" with 4 m^3 of the surrounding sea water drawn from a depth of about 4 m. This water was pumped through an all-plastic assembly, and was strained through 320-μ mesh nylon netting to remove all but the smaller zooplankters.

The initial water in the bag was entirely free from animals and contained few if any plant cells. The chlorophyll *a* content of about 0.15 mg/m^3 was probably detrital, although there could have been a few microflagellates present. The inoculum was high in pigment (an average chlorophyll *a* content of 11 mg/m^3) and undoubtedly introduced some animal population into the bag. However, no significant zooplankton population developed during the course of the experiment and no chitin was detected analytically at any time.

The initial "background" level of particulate organic matter was undesirably high. The material consisted mainly of protein and we cannot explain its presence. Most probably there was a leak of diatomaceous earth at one stage in the filling, or detrital material of near-colloidal dimensions may have escaped filtration. The amount was very much less than in unfiltered sea water.

The inorganic nutrient content of the bottom water used to fill the plastic sphere was large and representative of the winter levels of concentration found in this area, which is one of the most fertile coastal regions of the world. Therefore, no artificial fertilization with silicate, nitrate, or phosphate was attempted.

As a daily routine, the depth of the plastic sphere was adjusted to a constant level by the suitable addition or removal of air from the buoyancy bell. The temperature and salinity of the water at the center of the bag and at the same depth in the sea alongside the bag was measured first thing each morning. A plastic Nansen reversing bottle with protected thermometer was employed to measure temperature and to take samples for salinity determination.

At about 0900 hr each day samples were taken in a plastic Van Dorn bottle and used for water analyses. The water was filtered at once through a Millipore HA membrane filter and suitable aliquots of the filtrate stored at –20°C in the dark until ready for analysis. Separate samples were drawn for oxygen, pH and alkalinity determinations, which were performed immediately.

Measurements of the attenuation of light in the sea and in the bag were carried out in the mid-morning and mid-afternoon. At about 1030 hr an 8-L sample was taken in Van Dorn bottles and well mixed in a polyethylene aspirator. Nitrogen was passed through the water, if necessary, to reduce the concentration of dissolved oxygen to below the saturation level and then the water was passed into 300-ml B.O.D. bottles for the measurement of photosynthesis. Some bottles were placed in a stainless steel holder suspended at a point 2 ft above the center of the bag on the stirring line and were left in the bag for 24 hr (1200 to 1200). Other bottles were illuminated in a laboratory incubator under constant (artificial) light conditions for a period of less than 6 hr.

At 1330 the water in the bag was sampled for the analysis of particulate matter. Samples were taken at the mid-point of the plastic sphere, using a Van Dorn bottle, and were mixed and stored in a 20-L polyethylene bottle. Filtration of this sample was commenced without delay.

Occasionally samples were taken from several depths and analyzed for pigment and dissolved oxygen, *etc.* in order to check on the uniformity of distribution of metabolites within the sphere.

Determination of dissolved substances

Full details of all analytical methods are given by Strickland and Parsons (1961). The following brief summary indicates the general techniques that were used.

Salinity was measured by an electrical conductivity salinometer, oxygen by the Winkler method, pH using the Beckman

model GS meter and a glass electrode standardized by standard phosphate buffer at pH 6.87, alkalinity by the Anderson-Robinson method (Anderson and Robinson 1946), silicate by the method of Mullin and Riley (1955a), nitrite by the method of Bendschneider and Robinson (1952), nitrate by a modification of the procedure described by Mullin and Riley (1955b), and phosphate-phosphorus by a modification of the method of Robinson and Thompson (1948). Total soluble phosphorus was determined as phosphate after wet oxidation wtih perchloric acid (*cf.* Hansen and Robinson 1953). Soluble organic phosphorus was measured as the difference between the total phosphate in a sample passed through a Millipore HA filter and the inorganic phosphate in the same sample. Particulate and soluble reactive iron and ammonia were estimated as outlined by Strickland and Austin (1959). The method used for manganese was based on the catalyzed oxidation of the leucobase of malachite green by periodate, as developed by one of the present authors (see also Yuen 1958). Copper was determined as the colored diethyldithiocarbamate complex which was extracted directly into carbon tetrachloride. Soluble organic nitrogen was estimated by evaporating 25 ml of filtered water and subjecting the residue to Kjeldahl oxidation. The ammonia in the residue was then determined absorptiometrically, as above, and the result corrected for any ammonia found in the initial sample.

The change of total carbonate-carbon in the bag was obtained from precise measurements of the change in pH, knowing the total alkalinity of the system, and using a modification of the Buch tables given by Strickland and Parsons (1961) with graphical interpolations. As only changes in carbonate concentration were being measured in an isolated system, a very precise alkalinity determination was not necessary (*cf.* Strickland 1960).

Determination of particulate matter

Details of all methods (with the exception of the enumeration of phytoplankton cells) are described by Strickland and Parsons (1961). A brief summary of the procedures will be given here for the convenience of the reader.

All sea water samples were first filtered through a nylon net with a mesh size of 300 μ to remove any large particles of extraneous detritus introduced during sampling. The concentration of the particulate matter was accomplished by filtering the water through a Millipore type AA membrane filter, previously treated with magnesium carbonate (Parsons and Strickland 1959). When sufficient sea water had been filtered the Millipore filter was removed and the concentrated particulate material was washed from the surface with a small quantity of 3.0% saline. The suspended particulate matter and magnesium carbonate were then further concentrated by centrifugation and the supernatent liquid was discarded. The resulting residue was used for the determination of carbon, carbohydrate, crude fibre carbohydrate, protein, fat, nitrogen, and phosphorus.

Plant pigments

Chlorophyll *a*, *b*, *c* and carotenoids were measured as described by Richards with Thompson (1952) using the Millipore filter technique to concentrate the phytoplankton cells described by Creitz and Richards (1955). Extinctions were measured in 10-cm cuvettes. A correction for turbidity was made using the extinction at a wave length of 7,500 A, multiplied by a predetermined factor to allow for increasing light scatter at decreasing wave lengths.

Carbon

Particulate carbon was determined by wet oxidation with potassium dichromate in concentrated sulphuric acid by a modification of the procedure described by Johnson (1949). The decrease in the extinction of dichromate solutions at a wave length of 4,400 A served as a measure of the amount of oxidizable carbon.

Protein and nitrogen

Particulate protein was determined by two methods. The first involved Kjeldahl digestion of the sample and an estimation of the ammonia using Nessler's reagent, Johnson (1941). Nitrogen values determined by this

method were converted to protein values by multiplying by a factor of 6.25. The second method involved the hydrolysis of the particulate protein with 6 N HCl at 100°C for 6 hr, followed by a colorimetric determination of the hydrolyzed protein using 2,5-hexanedione and Ehrlich's reagent, as described by Keeler (1959). The method was standardized using pure dry casein. Nitrogen values were obtained by dividing by a factor of 6.35. Unlike the tyrosine methods for protein, the Keeler technique involves the reaction of 8 amino acids and ammonia in a hydrolysate. However, results still depend on the amino acid spectrum of the algal proteins which differs from species to species and which cannot be expected to resemble that of casein exactly.

Carbohydrate

Particulate carbohydrate was determined by the anthrone reaction using the reagents employed by Hewitt (1958) and measuring the light absorption of the colored solution developed at a wave length of 6,200 A. Additional absorptions were measured at wave lengths of 5,500 A and 6,500 A in order to determine, qualitatively, the presence of hexuronic acids and pentoses. The method was standardized with glucose. "Crude Fibre Carbohydrate" was also determined by the anthrone reagent on a concentrated residue after consecutive treatments with 1.25% sulphuric acid and 1.25% sodium hydroxide in a boiling water bath.

Fat

The amount of fat present in the particulate matter was determined by a modification of the method described by Mukerjee (1956). The method specifically measures fatty acids. Results obtained by the use of this method may be less than values obtained by an ether extraction technique. The method was standardized with stearic acid.

Particulate phosphorus

Particulate phosphorus was determined as phosphate after a wet oxidation with perchloric acid (*cf.* Hansen and Robinson 1953). The particulate phosphorus is defined as that retained on a Millipore HA filter.

Enumeration of phytoplankton cells

Phytoplankton cells were counted using a Unitron inverted biological microscope. Samples of sea water were preserved in 2% neutral formalin and counts were made on 5- or 10-ml aliquots of sea water without prior concentration of the cells. Samples were counted at magnifications of 50×, 100×, and 400×. Duplicate counts of 20 fields made at equal intervals across the diameter of the cylinders (2.6 cm in diameter) normally agreed within 10%. An approximate estimate of the average cell volume of each of the principal species present was made by direct microscopical measurement of the dimensions and shapes of the cells. All cells were counted, living or dead, provided that they were sufficiently intact for ready identification.

Phytoplankton cell numbers, as such, were of limited interest because of the enormous size differences between one species and another. For better comparison total cell numbers were converted to total cell volume (μ^3) by the following experimentally determined factors: *Nitzschia delicatessima*, 500; *Skeletonema costatum*, 1,900; *Glenodinium danicum*, 3,600; *Thalassiosira nordenskiöldii*, 11,000; *Thalassiosira rotula*, 35,000, and *Gyrodinium fulvum*(?), 150,000. (In the other experiment, of only 5 days duration, the following additional factors were used: *Coscinodiscus radiatus*, 12,500; *Coscinodiscus centralis*, 7,350,000.) To convert the resulting algal volumes to organic carbon a factor of 0.1 was employed (*cf.* Strickland 1960). The evaluation of total carbon from cell counts by the above procedure is, at best, very approximate. However, results of cell counts alone can be most misleading unless some such attempt is made to translate cell numbers into a measure of biomass.

Measurement of radiant energy

Incident radiation was measured with an Eppley pyrheliometer situated on a hill about 400 yd from the plastic sphere. Photosynthetically active radiation (3,800–7,200 A) was assumed to be 50% of that measured by the pyrheliometer, and is reported in cal/cm²/min (ly/min, *cf.* Strickland 1958).

Mean daily light intensities are the averages of the values recorded between sunrise and sunset.

Optical extinction coefficients of the water in the bag and the surrounding sea were determined from measurements with an underwater light meter and deck cell. Both cells were fitted with 2 mm of Schott BG. 12 blue glass, giving a maximum response of photocell-filter combination near to a wave length of 4,300 A. The attenuation of photosynthetically active light was estimated from these extinction coefficients using the data collected by Jerlov (1951).

Mean daily light intensities at the center of the plastic bag were calculated using the extinction coefficient of the surrounding water through the first 3 m, and then the extinction coefficient of the water in the sphere through a further 3 m. When the morning and afternoon extinction coefficients differed markedly their mean value was used. It must be emphasized that the radiation intensities obtained by this method are only very approximate but relative values from day to day are strictly comparable.

Measurement of photosynthesis

Measurements of photosynthesis were carried out by both the light and dark bottle oxygen method and a modification of Nielsen's C^{14} technique (see Strickland and Parsons 1961). All rates were measured using duplicate "light" bottles and a single "dark" bottle. The initial oxygen concentration was determined so that the net oxygen changes and respiration values in dark bottles could be evaluated.

The constant light intensity incubator used in this work held the B.O.D. bottles lying horizontally in black painted trays and cooled by running tap water. Samples were illuminated from above by blue and green fluorescent lamps emitting 0.1 ly/min of radiation as measured by a pyrheliometer.

No account was taken of possible periodicity effects in photosynthesis as these are thought to be quite small at latitude 49°N (Doty 1959).

One would suppose from the work of Fogg (1958) and others that $C^{14}O_2$ uptake experiments of more than about 6-hr duration would give errors due to the secretion of labelled material. It was impracticable, however, to conduct a series of short exposures in the sphere throughout each day and extrapolation from a single 6-hr period to a full 24 hr of light and dark was too inaccurate to be of use. Therefore we resorted to 24-hr radiocarbon experiments, on the assumption that at low light intensities the results would be tolerably accurate, as already reported by Rodhe (1958). We confirmed this experimentally by comparing the sum of the uptakes in two half-day exposures with that of a full 24-hr exposure in the bag. At the higher light intensity in the incubator there was very poor agreement between the rates found with 6- and 24-hr exposures, again supporting the findings of Rodhe (1958). At this higher light intensity plants were metabolizing many times more rapidly in the incubator than in the bag, and dividing once or twice in the 24-hr period of continuous illumination. Under such conditions the secretion of metabolites and "light fatigue" of the plant cells would be expected to be pronounced.

Experiments to measure the relative rate of photosynthesis as a function of illumination were carried out with the C^{14} method and the wire-screen attenuators described by McAllister and Strickland (1961). Natural daylight was used as a source of illumination during the period between about 1000 and 1400 on cloudless days. The light intensity over this period was nearly constant.

RESULTS

The salinity of the water in the bag was 27.00‰ and its temperature initially 11°C. The transmission of light through the polyvinyl chloride plastic and its supporting coarse-mesh nylon netting was greater than 75%, excluding any effects from direct shading by the supporting buoyancy bell and cradle. Temperature changes in the water inside the sphere closely followed those of the surrounding sea water. This is illustrated by Figure 1 which shows the total daily amount of solar radiation at the sea surface, the temperature at 5 m depth in the sea, and

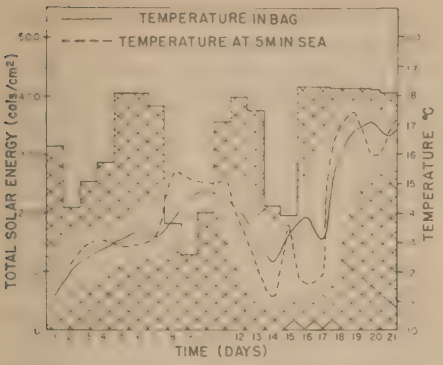

FIG 1. Total solar radiation and the water temperature inside and outside the plastic sphere.

the temperature at 5.5 m in the bag. It is interesting to note the delay of some 4 days before the sea temperature showed the influence of large changes of incoming radiation. The temperature of the water in the bag followed that of the sea outside with a lag of only about one day.

Most of the data obtained during the present experiment are summarized graphically, and generally the limits of variation are indicated by vertical lines or by the size of the plotted points. The confidence limits embrace the 95% probability region or are thought to approximate this value. Certain important ratios of metabolites and of metabolites to photosynthetic rates, *etc.* are collected in the tables and will be discussed later. The precision of these ratios varies greatly but their significance may generally be judged by the number of significant figures quoted. Some other results appear to have little obvious significance and will be summarized briefly in this section.

No chlorophyll *b* was detected in any analyses and the level of animal carotenoid (astacin) pigments was very low and erratic. The apparent presence of the latter may have been an analytical artifact. Copper was present in a concentration of 0.048 μg-at Cu/L which did not change throughout the three weeks of the experiment. The concentration of reactive manganese varied between 0.01 and 0.04 μg-at Mn/L with little obvious pattern, although there was a maximum just before the main phytoplankton growth period started (day 8). Soluble reactive iron changed little in concentration being in the range 0.07 to 0.12 μg-at Fe/L. The amount of particulate reactive iron was quite large. Initially present at 0.7 μg-at Fe/L it decreased in quantity to one-third of this value by day 12. The concentration thereafter increased to the initial level. The significance of this behavior, if any, is not clear. The decrease roughly paralleled that of soluble nitrogen but more experimental work is necessary before definite conclusions are possible.

The initial concentration of soluble organic phosphorus in the sphere was appreciable (0.3 μg-at/L) and seems to have increased slowly during the growth of the phytoplankton crop and then more rapidly as the crop decayed, reaching a value as high as 0.55 μg-at/L. Three anomalous results were obtained in this series of determinations and more work is required before any definite pattern can be stated. The concentration of soluble organic nitrogen showed a similar increase throughout the experiment (about 2 up to 7 μg-at N/L) but there was no marked correlation with the algal growth behavior. The precision of this method is relatively low (±0.8 μg-at N/L or worse) which may have masked any subtle relationships.

In a well-stirred conserved system the decrease of total dissolved nitrogen or phosphorus must be equal to the increase found in the particulate matter. The analytical results of losses and gains in the present work gave tolerable agreement until after day 17. Then serious discrepancies resulted which showed that stirring was insufficient to keep dead and dying cells in suspension. The decrease of particulate matter after day 17, therefore, represents both the true dissolution of solids and their removal from the bag center by settling. Although analytical results on the particulate material, *per se*, are still valid, no conclusions can be drawn from *changes* of concentration (as sampled at the center of sphere) for the last 5 days of the experiment.

The useful life of the plastic bag was ter-

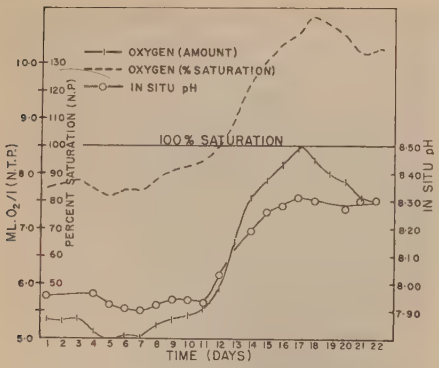

FIG. 2. Changes in pH and dissolved oxygen.

minated by tears near the neck (at day 5 in one trial and day 23 in the main experiment). As the salinity of the surrounding sea water was generally 2 or 3‰ less than the water in the bag the entry of outside water was easily detected by precise (conductivity) salinity measurements.

DISCUSSION

The net production of organic matter during the course of the main experiment is illustrated by Figure 2, which records the change of pH and oxygen concentration in the plastic sphere. The percentage saturation of oxygen (at atmospheric pressure) shows the striking degree of supersaturation often reported in the literature and attributed to the effect of photosynthesis. A value of nearly 150% was recorded in the present work. The subsequent decrease of oxygen concentration (after day 17) was mainly due to physical desaturation processes as no corresponding decrease of pH occurred.

The main phytoplankton species present at various stages in the experiment are shown in Figure 3. The use of algal weight (derived from algal volume assuming a mean density of unity) enables one to comprehend the relative contribution of each species to the overall primary production. The total cell count exceeded that of the named species by up to 25% but the unnamed species represented a much smaller addition to the biomass than numbers alone would indicate. *Asterionella japonica* was the most conspicuous of the minor constituents.

The growth rate of each species was not uniform but over the period of most constant growth mean division times could be evaluated. Values for the diatoms *Skeletonema costatum*, *Thalassiosira rotula*, *Thalassiosira nordenskiöldii*, and the dinoflagellate *Glenodinium danicum* were very similar (30–38 hr). The dinoflagellate *Gyrodinium fulvum* (?) was noticeably more rapid in growth (22 hr) and for an initial period *T. rotula* increased with a doubling time of only 19 hr. The cell numbers of all species eventually showed a decrease which we attribute to the rapid sinking of dead or dying cells (ref., *e.g.*, Gross and Zeuthen 1948; Steele and Yentsch 1960). *T. rotula* passed its peak of metabolic efficiency somewhere between days 8 and 11. This could reflect either a marked sensitivity to the concentration of nitrate or be the result of an increase of water temperature from 12 to 15°C. One cannot rule out the possibility of an inhibition by the increasing concentration of *Skeletonema* cells. *Nitzschia delicatessima* was the only organism to grow to any degree under markedly unfavorable conditions (a doubling time of over 80 hr) and this species commenced to die off soon after the depletion of nitrate at the end of day 13 (Fig. 4). *Asterionella japonica* persisted in small numbers throughout most of the experiment but was obviously living under conditions so unfavorable as to bring cell division almost to a standstill.

The growth rate of *S. costatum* decreased as soon as nitrate disappeared from solution but, statistically, the cells were capable of one further division. *T. nordenskiöldii* grew more vigorously and divided twice before the cells commenced to die. By contrast the two dinoflagellates only began to increase rapidly after the nitrate was exhausted and *Gyrodinium fulvum* survived in fair condition until the end of the experiment. The above observations give a striking illustration of the kind of conditions that bring about a "succession of species" in nature (*cf.* Conover 1956; Margalef 1956). We are unable to explain the kinks in the curves for *T.*

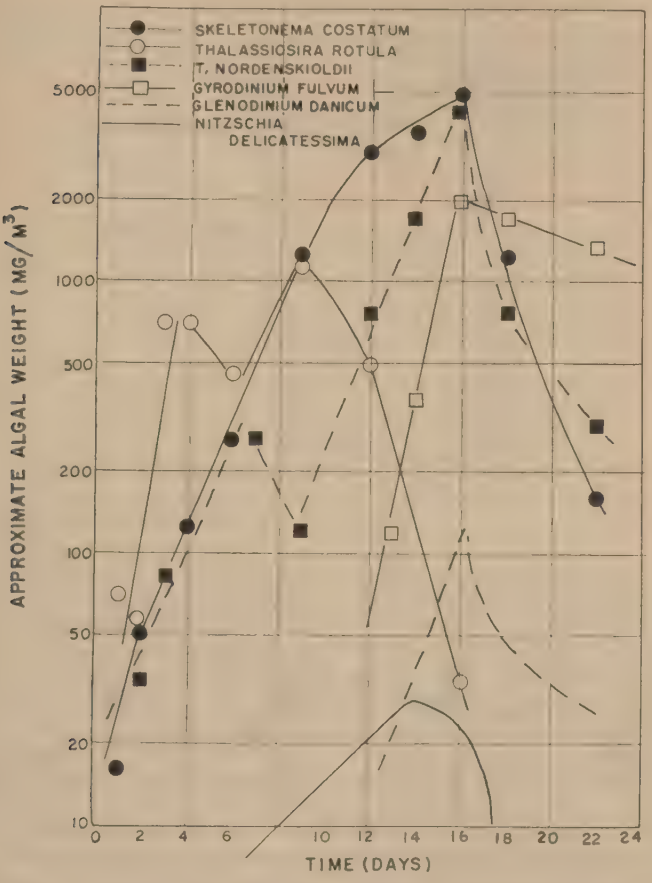

FIG. 3. Changes in the algal weight of the main phytoplankton species.

rotula and *T. nordenskiöldii* (there was a similar very high point for *Skeletonema* at day 3, not plotted). There must have been an error either in sampling or in counting brought about by a change in mean chain length, and hence a contagion factor, for the species concerned.

Iron, copper, and manganese were clearly not limiting nutrients for plant growth and reactive silicate was initially present at such a concentration that it never became depleted, despite the crop being predominantly diatoms. One would infer from our results that the initial Si:N ratio in sea water would have to be less than 2:1 (on an atom basis) for silicon to become a limiting nutrient in a diatom bloom. It is interesting to note the small but significant rise in silicate concentration after day 16 which indicates that a fraction of the silicon in a diatom is very labile (*cf.* Jørgensen 1955a). Subsequently there was only very slow remineralization. In considering this fact and the remineralization of nitrogen (see later) it must be remembered that the present experiment is unrepresentative of nature in that no regeneration processes could occur as a result of grazing (*cf.* Cushing 1959).

The initial ratio of nitrogen to phosphorus in solution was 9 (on an atom basis) or 10 if

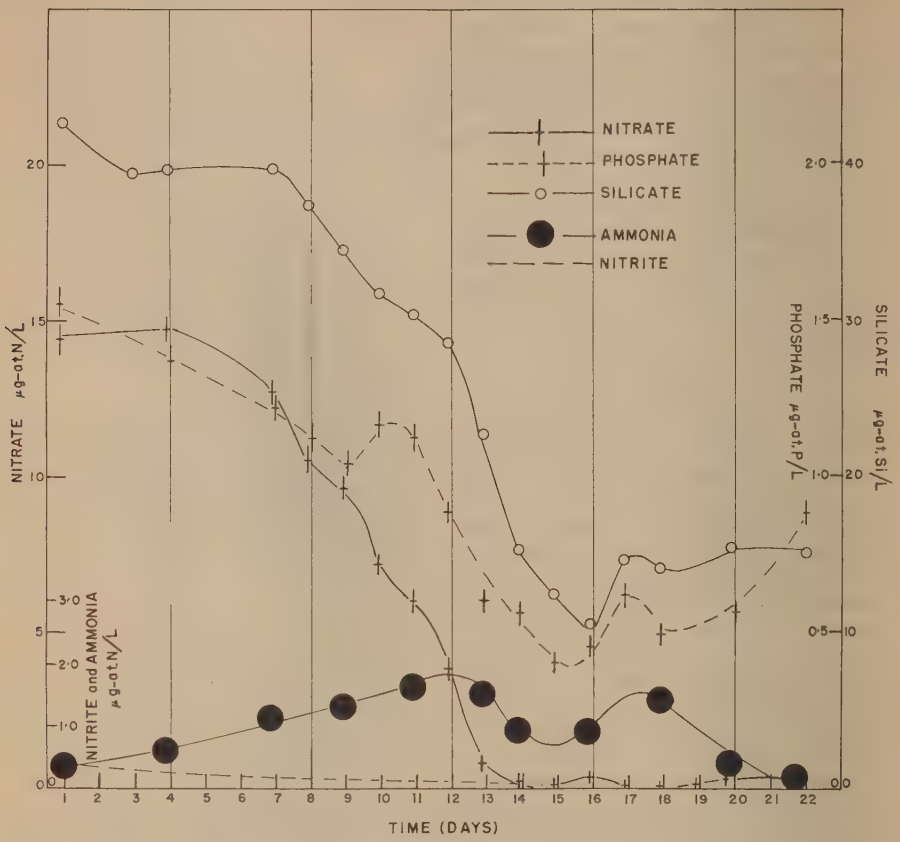

FIG. 4. Changes in the concentration of nitrate, nitrite, ammonia, reactive phosphate and reactive silicate.

we consider only nitrate and inorganic phosphate. On the assumption that growing plants withdraw these elements from sea water in the mean atomic ratio of 15:1 (Cooper 1937, 1938) one would expect to find nitrate removed before phosphate, as was indeed the case (Fig. 4). Furthermore, the tendency for phosphate to continue to decrease in concentration after all the nitrate has gone, as found in cultures of open Pacific Ocean water by McAllister, Parsons, and Strickland (1960), was also shown in this experiment with coastal water. It will be seen from Figure 4 and from the analytical data given in Table 2, that the ratio of nitrogen to phosphorus in the growing cells was variable, being quite low during the first part of the experiment and then increasing to approach the "classical" 15:1 value.

Phosphorus was not a "limiting nutrient." The comparatively labile nature of its incorporation in phytoplankton (Gest and Kamen 1948; Goldberg, Walker and Whisenand 1951; Rice 1953; Rigler 1956) and the rapidity with which it can leave dead cells (Hoffmann 1956; Golterman 1960) is reflected by irregularities in the curve in Figure 4. In particular there was a small but definite *increase* in the amount of the element in solution between days 9 and 10. Cells appear to have stored an abnormally large amount of phosphorus (see Table 2), some of which

TABLE 1. *Ratios involving photosynthetic rates*

Day	Ratio: Rate. (O_2 gross) / Rate. (C^{14}) (PQ=1)		Ratio: Rate. mg C/hr (O_2 gross) / mg chlorophyll *a* (PQ=1.3)	Productivity index[2] from gross O_2 (PQ=1.3)
	Bag	Incubator	Incubator	Incubator
1	–	–	1.3[1]	–
2	–	–	1.2[1]	–
5	–	3.3	1.65	–
6	2.4	–	–	–
7	1.8	–	–	–
8	2.1	3.3	1.6	–
9	2.7	–	–	–
10	1.9	2.9	1.75	0.9
11	1.9	3.3	1.6	0.75
12	1.9	3.3 *Mean 3.3*	1.45	0.75
14	1.7	–	1.6	0.75
15	2.2	2.0	1.65	0.75
16	1.5	1.7	1.05	0.55
17	2.0	1.4	1.05	0.4
18	1.5	1.9 *Mean 1.75*	0.95	0.2
19	1.7 *Mean 1.95*	–	–	–

[1] Values using carbon-14 rate measurements.
[2] Carbon measured by direct chemical method.

was then liberated during a period of low illumination and increasing temperature. Phosphorus was obviously lost more rapidly than nitrogen from dead cells and this is also shown by the ratios given in Table 2.

Nitrite nitrogen, initially present at 0.35 μg-at N/L decreased to one-fifth of this value in the first 14 days. It is not possible to say whether or not nitrite was incorporated into phytoplankton (*cf.* Harvey 1953; Bongers 1956) or oxidized by bacteria.

Whereas nitrate nitrogen was consumed rapidly and in a more or less regular fashion it is interesting to see that there was an increase in ammonia concentration (Fig. 4) until day 13 and a significant increase in the amount of soluble organic nitrogen. Ammonia was clearly not used in preference to nitrate when the latter was in great excess (*cf.* Harvey 1940) although when nitrate became depleted there was some indication that ammonia could be scavenged from solution and might, in this instance, have been providing *Gyrodinium fulvum* with its most readily accessible supply of nitrogen. There was little or no indication of any nitrogen remineralization, but this is not surprising considering the time scale suggested by the work of Von Brand, Rakestraw and Renn (1939), Spencer (1956) and others and the fact that some plant cells were still living and removing available forms of the element until the end of the experiment.

As noted by several workers (*e.g.*, Harvey 1953; Rodhe 1957) the growth rate of phytoplankton can be quite rapid even when all nitrate has been removed from a medium and cells exist for one or more divisions on their reserves of protein. The present work gives striking illustration of this fact as nearly half of the final organic matter in the phytoplankton bloom was produced after nitrate depletion. Any attempt to predict the kinetics of natural population growth using the concept of a "limiting concentration" of nitrogen (or phosphorus) therefore has little meaning unless the past history of the phytoplankton crop is also known. There may, of course, be conditions where the supply of nutrients is just balancing consumption but for how long such an unstable equilibrium could exist in nature, especially in coastal regions, remains to be shown.

There is a shortage in the literature of direct comparisons between the oxygen and C^{14} methods of measuring photosynthesis and many of the results quoted are for pure cultures. In the present work ratios were

measured by the two methods whenever practical, both at the center of the plastic bag for 24 hr and in the constant light incubator for shorter periods. In Table 1 the ratios between *gross* oxygen liberation and radioactive carbon uptake are presented with no assumptions as to what is being measured.

The ratios are disconcertingly large, but they can be reduced if a large photosynthetic quotient (*PQ*) is allowed and if the $C^{14}O_2$ method is assumed to measure net photosynthesis in cells which have a high respiration rate. The highest *PQ* and respiration values normally used are about 1.35 and 15% respectively which would give a ratio of 1.6 at moderate light intensities. (This subject is fully reviewed by Strickland 1960.)

The ratios measured by the bottles at the center of the bag averaged 1.95. They fell into two distinct groups in the incubator, where there was from 3 to 10 times more illumination. Values before day 14 (and nitrate depletion) averaged 3.3 and the four values after day 14 had a mean of 1.75. It is generally supposed that nitrogen deficient cells have a high respiration rate and hence low rate of net photosynthesis (Ketchum, *et al.* 1958) but these last four results do not indicate that the C^{14} results are usually low, unless *PQ* values are also very small. The ratio of 3.3 in a nitrate rich medium is even less easy to explain. One is compelled to assume very high values for the *PQ* of diatom cells taken from a nitrate rich medium at low illumination and exposed for a few hours to comparatively high light intensities. The whole question of a correct *PQ* value is of much greater importance in marine studies than is generally admitted, especially if one wishes to make an accurate estimate of carbon uptake from oxygen measurements.

It is interesting to note that the depletion of nitrate had no significant effect on the 24-hr ratios determined in the plastic bag. These values may be slightly high due to the prolonged period of $C^{14}O_2$ uptake.

With all these results we suspect that ratios are greater than they should be, even having regards to likely experimental errors and high *PQ* and respiration values. It is possible that in coastal water the C^{14} method is giving low results due to the photosynthetic assimilation of non-labeled forms of carbon (Smith, Tatsumoto and Hood 1960; and see also Strickland 1960 for a discussion).

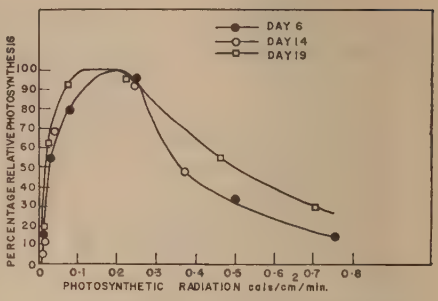

FIG. 5. Relative rate of C^{14} assimilation as a function of radiation intensity.

The relative photosynthetic rates (*f*) *vs.* illumination values (*I*) for the populations in the sphere on days 6 and 14 have been plotted on the same curve in Figure 5. This is very similar to that given by Ryther (1956). At day 19, when *Gyrodinium fulvum* comprised about half the population, the values lay on a slightly different curve. There was then the greater tolerance to high light intensities to be expected from a dinoflagellate (*e.g.*, Ryther 1956) but the curve at lower intensities still resembled that for the diatom population. It will be seen that the light intensity of 0.1 ly/min used in the incubator was at approximately the optimum value. Mean light intensities at the center of the bag did not exceed 0.055 ly/min and photosynthesis was therefore always roughly proportional to light intensity.

Back extrapolation of *f vs. I* curves to zero light intensity should indicate the amount of plant respiration, if the assumptions made by Nielsen and Hansen (1959) are justified. The values so obtained, about 10%, are reasonable.

Figure 6 shows the increase in carbon in the plastic bag from day 1 to day 22, as measured by both oxygen and C^{14} methods, again with no assumption of *PQ* values or the significance of the C^{14} measurements. The increase on day 13, and hence the total for all subsequent days, may be low as there

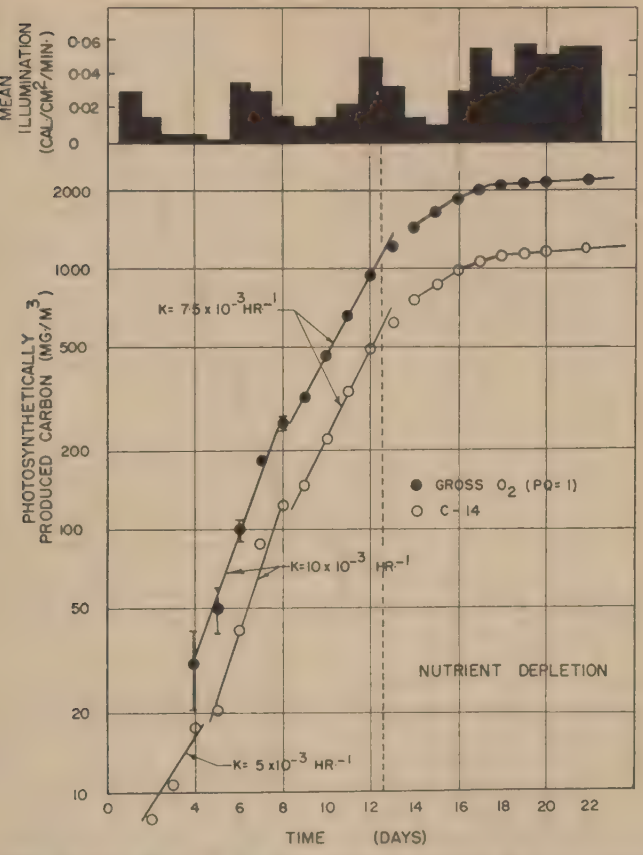

FIG. 6. Photosynthetic carbon fixation in the plastic sphere as a function of time under natural lighting.

was poor stirring that day with a temporary loss of plant cells. The curves are parallel and their slope can be used to evaluate logarithmic growth constants. The values given in Figure 6 (base 10) probably reflect changes in temperature and light intensity (see the histogram for mean daily illumination at the bag center). After the end of day 15, severe nitrogen shortage brought about a reduced growth rate and results no longer have much meaning, because cells were sinking below the sampling point in the bag.

The most fruitful way to examine the effect of nutritional changes on the photosynthetic metabolism of algal cells is to expose them to constant light intensity in an incubator and find either the rate of carbon increase per unit of chlorophyll *a*, or the productivity index, defined by Strickland (1960) as:

$$\frac{\text{hourly rate of carbon increase}}{(\text{amount of plant carbon}) \times (\text{illumination in ly/min})}$$

Both these values are given in Table 1, using the gross photosynthetic rate measured by oxygen evolution and an assumed *PQ* of 1.3. There was no significant change in the photosynthetic potential of the cells from day 1 to day 15, having regards to the general magnitude of experimental errors.

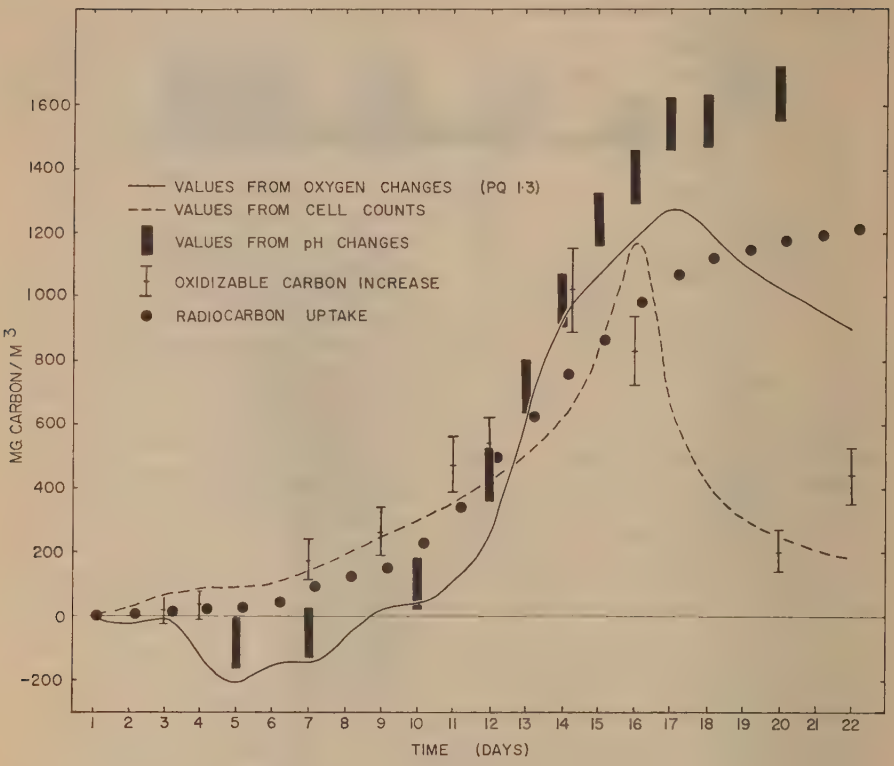

FIG. 7 Net production of carbon as measured by five independent methods.

The gross rates, per unit of chlorophyll *a* at optimum lighting, were very much lower than the rates reported by Ryther and Yentsch (1957), although they were within the range found in the literature (Strickland 1960) and comparable to the values found by McAllister, *et al.* (1960) in the open Pacific Ocean.

These productivity index values are the first of their kind to be reported, and are near to many of the values inferred from the literature (Strickland 1960). The high value at day 10 may be significant but was not reflected in the corresponding figure for the rate per unit of chlorophyll *a*. Similarly the decrease of productivity index at the end of the experiment was much more pronounced than the corresponding decrease in the chlorophyll ratios, but this would be expected because a large amount of detrital carbon was accumulating. In particular it should be noted that the loss of nitrate at the end of day 13 had no effect on either ratio. Only at day 16, when the mean nitrogen content per cell was probably less than a half of its peak value, did the photosynthetic processes, as reflected by these ratios, become seriously impaired. At the same time cells commenced to settle out rapidly (Fig. 3).

There was no evidence in our experiment for the situation reported by Ryther, *et al.* (1958) where the growth of a diatom bloom in nitrogen deficient water was static as the result of a sizable amount of photosynthesis during the day being compensated for by respiration during the hours of darkness.

The net production of organic particulate carbon in the plastic bag was estimated by

five methods and the results are shown together for comparison in Figure 7. The full line shows the values calculated from changes of dissolved oxygen in the bag, assuming a *PQ* of 1.3. Results are probably low after day 15 or 16 due to physical losses of oxygen from the supersaturated system. The dotted line is a measure of plant carbon derived from cell counts and, as mentioned earlier, is only very approximate. The bars show the range of individual results estimated from changes of total carbon dioxide content. This is the most direct method of all but unfortunately not as precise as could be wished. The solid circles mark uncorrected data calculated from the uptake of radiocarbon. These results were used in preference to the gross oxygen photosynthesis data because they formed a more complete set, and did not involve assumptions as to respiration rates and photosynthetic quotients. However, it is by no means certain that C^{14} data represent true net photosynthesis; they may well be too low. Finally, there are the experimentally measured oxidizable carbon results corrected for initial detritus. These results are entered as horizontal dashes with the 95% confidence limits indicated by vertical lines. Any excretion of soluble organic compounds during plant growth would make these results lower than they should be and this would also influence the ratios in Table 2.

Ignoring the measurements derived from dissolved oxygen and pH, the agreement between the other three estimates was as good as could be expected for much of the time. The two direct methods, carbon analysis and algal volume, finally showed decreases due to the settling of cells, although there was appreciable net synthesis going on inside the bag until at least day 20.

The various measurements of carbon during the first few days of this experiment gave the most anomalous results and they merit closer investigation. The dissolved oxygen content of the bag first *decreased*, representing the consumption of over 200 mg C/m^3, but there was no corresponding decrease of particulate carbon. A wall effect would not be expected so soon in equipment of this size (in the other experiment the oxygen decreased steadily from the first day) so presumably, much of the oxidation was by nonproliferating bacteria in suspension, using a substrate of dissolved organic carbon. This was borne out by the high but fairly constant respiration values found in the dark bottles used in photosynthesis experiments.

Not only was there no decrease of particulate matter, corresponding to the net consumption of oxygen in the bag, but the increase in photoplankton carbon, as estimated by cell volumes and changes of chlorophyll (see later) was appreciable and very rapid during the first 4 to 5 days. This increase, mainly in *T. rotula* and *Skeletonema*, could not be accounted for by photosynthesis, as the radio carbon uptake data predicted very much smaller values for carbon (one quarter or less). The most likely explanation, therefore, is that during the period of very low illumination at the start of this experiment (see Fig. 6) heterotrophic growth from dissolved organic carbon was a large fraction of the total growth. The effect was not detected in the 5 days of the second experiment, when the illumination was quite high. But at the beginning of the main experiment, a very heavy bloom of flagellates in the surrounding water prevented light entering the bag for several days. It is interesting to note that what little photosynthesis did occur during this period took place in a normal manner. The photosynthetic rate, per unit of chlorophyll *a*, in the incubator had about the same value as found during the rest of the experiment (Table 1).

Heterotrophic growth of algae is well established (Saunders 1957) and has been studied in marine diatoms by Lewin and Lewin (1960). Conversion of dissolved substrate to algal carbon can be high if the behavior of *Chlorella* is representative (Myers and Johnston 1949; Samejima and Myers 1958). Several workers have stressed the probable importance of heterotrophy in phytoplankters (Rodhe 1955; Wood 1956; Bernard 1958) but we believe the present work to be the most direct demonstration of its occurrence in marine phytoplankton under

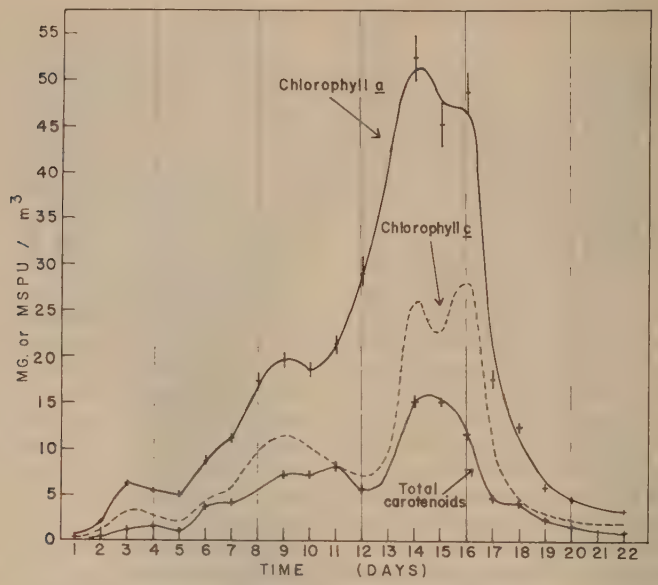

FIG. 8. Changes in the concentration of plant pigments.

conditions resembling those found at the base of the euphotic zone.

Variations of chlorophyll *a*, chlorophyll *c*, and total carotenoid concentration at the center of the bag are shown in Figure 8. The chlorophyll *c* curve roughly parallels that for chlorophyll *a* for most of the experiment, giving values about 50–60% of the chlorophyll *a*, in terms of MSP units and milligrams respectively. Between days 11 and 13, however, the ratio of chlorophyll *c* to chlorophyll *a* was much smaller, decreasing to 25%. This period coincided with the predominance of *Skeletonema costatum* in the bloom and we have found (unpublished work using pure cultures) that this species has what is probably an abnormally high chlorophyll *a* content and a low chlorophyll *c* to chlorophyll *a* ratio for a diatom. The large chlorophyll *a* content of *Skeletonema* would explain the very high maximum value found in the present experiment (50 mg/m^3). In the open ocean, a natural culture in water of essentially the same initial nitrate concentration bloomed to only 26 mg/m^3 chlorophyll *a* at its peak (McAllister, Parsons and Strickland 1960).

The first few days of the experiment are particularly interesting. In 3 days the chlorophyll *a* concentration increased from 0.4 to 6 mg/m^3. If we estimate carbon from cell volumes, the ratio of carbon to chlorophyll *a* is found to change from 25 to 11 in the first 3 days, which is reasonable for a surface population recovering its pigment content in a nutrient-rich, relatively dark environment. The remainder of the chlorophyll increase must be associated with a rapid development of cells during this initial period. Ratios calculated using photosynthetically produced carbon data were impossibly low which, as mentioned earlier, supports the suggestion of some heterotrophic growth.

The sudden decrease of chlorophyll after day 16 was striking and much more than would be expected from a decrease of cell numbers alone. The possible presence of "detrital chlorophyll" in nature is a matter of some importance in field studies. Odum, McConnel and Abbott (1959) could find no

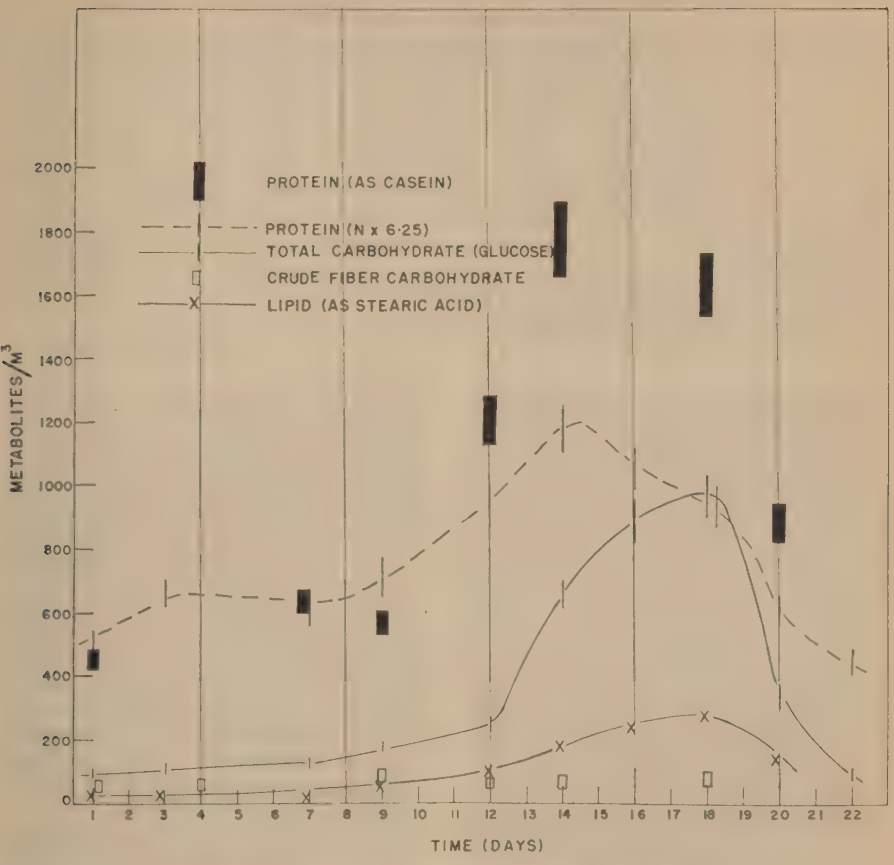

FIG. 9. Changes in the concentration of major metabolites.

spectrophotometric indication of such material. The present experiment seemed to be an excellent opportunity to investigate the matter. We measured the complete pigment spectrum, in 90% acetone, of the populations on day 12 when cells were growing with full vigor and day 18 when many cells were dead or dying but found no evidence for phaeophytin or phaeophorbide pigments. There was a noticeable broadening of the long-wave-length chlorophyll *a* peak in the extract made on day 18, and a slight shift to shorter wave lengths (6,650 A to 6,630 A). We have seen this effect in old laboratory cultures and it is reminiscent of the *in vivo* behavior reported by French and Elliott (1958). There is little likelihood, however, that such small changes would have any practical significance in marine pigment analysis. However, chlorophyllides, which could conceivably occur in decomposing plant tissue, have similar absorption spectra to chlorophylls and it also remains to be proven whether the detrital chlorophyll from faecal pellets is in the form of phaeopigments and hence may give misleading results in nature.

The composition of the particulate matter in the sphere, in terms of major metabolites, is summarized in Figure 9. In order to esti-

TABLE 2. *Ratios involving carbon*

Day	$\frac{Si^1}{C}$	$\frac{Protein}{C}$	$\frac{Protein^2}{C}$	$\frac{Carbohydrate}{C}$	$\frac{Lipid}{C}$	$\frac{C}{P}$	$\frac{C}{N}$	$\frac{C}{N^2}$	$\frac{N^2 \text{ (atoms)}}{P}$
7	–	0.66	0.89	0.2	–	19	9.5	7	6
9	1.0	0.72	0.76	0.3	–	17	9	8.5	5
12	0.6	0.78	1.4	0.3	0.15	23	8	4.5	11[3]
15	0.65	0.65	1.3	0.7	0.2	28	10	5	12.5[3]
18	–	0.65	1.8	1.35	0.4	40	10	4	25
20	–	0.43	1.9	0.7	0.55	35	15	3.5	23
Value suggested by Strickland (1960)	0.8	–	–	–	–	40±15	–	6±2	14.5

[1] Calculated from the decrease of reactive silicate.
[2] Values from the absorptiometric protein method, standardized with casein.
[3] The ratio had a maximum of about 15 on day 13.

mate the amounts of carbon, protein, carbohydrate, phosphorus, *etc.* in living phytoplankton it was necessary to subtract from the amounts found on a given day, the amounts of each metabolite present initially in the water used to fill the sphere and, further, to assume that this initial "background" concentration of detrital material did not change throughout the experiment. Almost certainly such an assumption is not fully justified, although there is reason to suppose that settling of detritus was comparatively slow. Errors from this source were most significant in the earlier part of the experiment and ratios such as those given in Tables 2 and 3 had little precision until at least day 7 or 9.

There was a considerable discrepancy between the amount of protein obtained by the two chemical methods. Some of this difference undoubtedly arises because the amino acid spectrum of algal protein is not the same as that of casein, but we would not expect from our work on pure phytoplankton cultures (unpublished) that the differences would have been so great. Evidence from the "balance" between dissolved and particulate nitrogen in the bag pointed to the Kjeldahl nitrogen values being too low. A similar poor balance between nitrate lost from solution and nitrogen found in growing algae has been reported by Yentsch and Vaccaro (1958) but we are at a loss to explain the matter and more work is desirable. In the meantime, we have reported both sets of data in Figure 9, Table 2 and Table 3 and suggest that the colorimetric protein values are probably nearer to the "correct" results until at least day 12.

Crude fibre carbohydrate in the bag never exceeded a few per cent of the total phytoplankton carbohydrate. This confirms our (unpublished) findings for pure phytoplankton cultures and for natural coastal populations at the time of heavy diatom blooms. A quite different analysis is found for water containing much detritus, when as much as 80% of the total carbohydrate may be present as crude fibre. (There was about 60% in the detrital carbohydrate initially in the water of the present experiment.) An analysis for crude fibre is one of the best rapid chemical indicators for detritus, as distinct from living plant cells, in the sea.

The most striking feature shown by Figure 9 is the way in which the lipid and the carbohydrate (mainly glucose) in the plant cells increased when nitrate became depleted from solution on day 13 (*cf.* Spoehr and Milner 1949; Bongers 1956; Fogg 1957). Between about days 13 and 17 fats and sugars were almost the sole products of photosynthesis.

The classical conception of diatoms containing large reserves of fat is not borne out by the present work. When cells were in a dying condition in nutrient depleted water their lipid content was higher than earlier in the experiment, but was still less than 20% of the dry weight (see Table 2, assuming about one-third of the dry weight of a diatom is carbon). In the sea, near the surface, nitrate depletion doubtless occurs frequently and

TABLE 3. *Ratios involving chlorophyll a*

Day	C / chl. *a*	C / Carotenoids[2]	N / chl. *a*	N[1] / chl. *a*	P / chl. *a*	Carotenoids[2] / chl. *a*	Protein / chl. *a*	Protein[1] / chl. *a*	Carbohydrate / chl. *a*	Lipid / chl. *a*
7	15	43	1.6	2.2	0.81	0.36	10	14	3	–
9	13	37	1.5	1.6	0.80	0.36	11	10	4	–
12	19	100	2.4	4.2	0.83	0.19	15	27	5.5	3
15	22	70	2.2	4.5	0.78	0.31	14	28	15	4
18	56	160	5.9	16	1.4	0.36	37	100	76	22
20	49	150	3.3	15	1.4	0.33	21	93	33	27
Values suggested by Strickland (1960)	30	40	–	7±3	0.75±0.2	–	–	–	–	–

[1] Values from the absorptiometric protein method, standardized with casein.
[2] Total carotenoids in MSPU.

hence diatoms collected by net tows may often be expected to have their maximum lipid content (*cf.* Barker 1935). However, it is difficult to believe from the present work that the presence of fat assists diatoms in maintaining their buoyancy to any great extent.

The ratios of elements and metabolites to carbon and chlorophyll *a* at various stages in the phytoplankton bloom are given in Tables 2 and 3 respectively. The ratios involving carbon (the direct oxidizable carbon data were used) illustrate the relative changes in composition of the phytoplankton with time. The values involving chlorophyll *a* are important mainly because this pigment is the only convenient measure of plant material in sea water, as distinct from animals and detritus. Data such as those in Table 3 show the limits within which the plant carbon, nitrogen, metabolites, *etc.* can be estimated, knowing the pigment content of a water sample. Clearly each possible mixed algal population in nature, at every stage of its growth, will give rise to different ratios so that the value of any particular one is limited. Nevertheless, as pointed out by Strickland (1960) there are still very few relevant data of this kind available for marine phytoplankton. The results given here are a useful beginning, especially as they apply to a mixed diatom population growing under near-natural conditions. For comparison, the approximate mean values for marine crops suggested by Strickland (1960) are given at the bottom of each table.

It is unfortunate that the precision of the ratios measured during the early part of the experiment is not as high as could be desired, but the data for days 7 and 9 are approximately correct for a diatom population growing under low illumination in nutrient rich waters. The data for day 12 apply to a vigorously growing and rapidly dividing bloom, about to strip the nutrients from the water. On day 15 the results apply to algae at the end of their growth period in nitrogen-deficient water. By day 18 there were many dead and dying cells, and on day 20 little living material was present at the bag center, except dinoflagellates.

The Si:C ratios given in Table 2 are some of the few to be reported for a live natural population and are of the order to be expected from results cited in the literature. The decrease from day 9 to day 12 may be real, supporting the observations made by Jørgensen (1955b).

Most of the other ratios in Tables 2 and 3 are self-explanatory and illustrate the trends that have already been discussed. The increase in the ratios involving chlorophyll *a* after day 15 reflects the rapid loss of this pigment from dead or dying plant material, a loss more nearly paralleled by that of phosphorus and total carotenoid, than by any other of the measured variables.

The co-variation of chlorophyll *a* and the carotenoids is indeed remarkable and supports the detailed observations made by Haskin (1941) on *Chlorella* and our own (unpublished) work on pure marine cultures.

Except for day 12, when there was a preponderance of *Skeletonema costatum*, which we have found to have a relatively low carotenoid content, the ratios were very constant and similar to the values found for mixed coastal populations in Departure Bay at all times of the year. There was no evidence of either the particulate nitrogen values or the ratios in Table 1 being functions of the carotenoid-to-chlorophyll ratio, as has been reported by Ketchum, *et al.* (1958) and Yentsch and Vaccaro (1958). Why there should be this discrepancy between their well-established laboratory results and the present large-scale experiment is not clear. Of course, conditions were not identical and this emphasizes the dangers of extrapolating from laboratory-scale results or even, to a lesser extent, from the present experiment to phenomena taking place in the sea under completely natural conditions.

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B. C. HERRING MANAGEMENT

By DR. F. H. C. TAYLOR

Management of the herring fishery in B.C. has been relatively successful. Stocks appear to be able to withstand even the current high levels of fishing intensity, but scientists say that "no large increases in catch other than those arising from the presence of large year-classes can be expected without endangering the stocks". This paper by the herring specialist of the Nanaimo Biological Station outlines the current status and management of B.C.'s second most important fishery.

In 1959 the world catch of herring amounted to 3,491,400 tons. Atlantic herring contributed 76%, Pacific herring 16%, and Baltic herring 8%. Of the total Pacific herring catch Russia took 258,830 tons, Canada 221,540 tons, the United States 53,680 tons, and Japan 18,590 tons. The 1959 North American herring catch of 275,000 tons was 1¾ times as great as the catch of any other fish landed at North American ports. Other catches were: salmon 153,340 tons; tuna 146,300 tons; groundfish 68,668 tons: halibut 35,800 tons; and sardine 35,364 tons. In value, however, herring ranked fourth with 9 million dollars, compared to 101 million dollars for salmon; 36.5 million dollars for tuna and 13.5 million dollars for halibut.

In British Columbia the catch of Pacific herring ranks first in landed weight and second to salmon in landed value. While the herring catch in recent years has averaged about three times the weight of the salmon catch, it has only been about one-quarter as valuable.

History

Herring have been fished in British Columbia waters since 1877, when a catch of about 75 tons was made. In 1955-56 there was a record catch of 250,960 tons. The general trend has been towards a steady expansion of the fishery, primarily in response to market conditions. The most important influences were the development between 1904 and 1927 of the Oriental market for drysalt herring, the loss of this market between 1927 and 1933-34 and the development starting in 1933-34 of the reduction fishery for oil and meal.

FIGURE 1: Major herring populations on the B.C. coast.

The expansion of the herring fishery from 1904 onwards was accomplished first through the increased exploitation of southern British Columbia stocks, followed in the late 1920's and 1930's by the expansion of the fishery northward, and finally since the end of World War II by a still further increased and heavy exploitation of almost all stocks.

Populations of Herring

Two main types of herring population are recognized in British Columbia, the main migratory populations and the resident or local populations found usually toward the heads of inlets or as "homesteaders" in certain other areas. These two types of population have been separated on the basis of differences in stock size, seasonal migration pattern, growth and age composition, location of spawning grounds, and similarity of runs forming the stock.

An analysis of tag recoveries has indicated that there are at least nine major migratory populations of herring in British Columbia (Figure 1). These are:

1. The upper Queen Charlotte Islands, centered in Skidegate Inlet.

2. The lower Queen Charlotte Islands—the east shore of Moresby Island with the main centre of the fishery in Burnaby Narrows.

3. The northern—Areas 3, 4 and 5.

4. The upper central—in Area 6, the west shore of Princess Royal Island from Barnard Cove to Meyers Pass.

5. The lower central—the outer portions of Areas 7 and 8.

6. The middle east coast of Vancouver Island—Areas 13-16.

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Dr. Fred Taylor, 42, received his BA from the University of B.C. in 1940. From 1941 to 1945 he served with the RCAF, returning after the war to a position at the Nanaimo Biological Station. He received his MA in 1947 from UBC. He was awarded his Ph.D. by the University of California—Scripps Institute of Oceanography in 1956.

He served on the staff of the International Pacific Salmon Fisheries Commission during the summers of 1939 and 1940. From 1945 onwards he has been associated with the Fisheries Research Board of Canada. From 1945 to 1947 he was connected with groundfish investigations, and in 1951 he was appointed scientific assistant to the director of the Nanaimo Biological Station, and was responsible for the Canadian contribution to the fur seal research leading up to the International North Pacific Fur Seal Commission. In 1954 he was placed in charge of herring investigations, and is presently carrying on this work.

Reprinted from "Western Fisheries" August, 1961.

FRB No. 683

7. The lower east coast of Vancouver Island—Areas 17A-19.

8. The lower west coast of Vancouver Island—Areas 23 and 24.

9. The upper west coast of Vancouver Island—Areas 25, 26 and 27.

Herring from the west coast of the Queen Charlotte Islands may form a tenth major population. However, very little is known about the herring from this region.

Regulation

The British Columbia herring fishery is regulated by a combination of closed areas, closed season and catch quotas. Closed areas are of little significance in limiting the catch, and are applied to certain small localities to protect local stocks for food or bait for natives or to allay fears of residents that large scale herring fishing might affect local salmon fishing. Closed seasons are of somewhat more significance in that if catch quotas are not taken, the season is closed during the actual spawning season. The closed season is from February 5 to June 1 in southern British Columbia and March 10 to June 1 in northern British Columbia. By mutual agreement, between the fishing companies and the fishermen's union, there is a 48-hour closed period each week end, and a three to four week closed period over Christmas. As conservation measures, these are of relatively little significance. Effective control of the herring fishery is provided by catch quotas on the various populations. This system is made flexible by quota extensions granted when the abundance of fish on the grounds warrants such action and by the authority of the Area Director to close the fishery at any time to conserve the stocks.

Herring fishing has been carried on in B.C. waters since 1877, when about 75 tons were taken. Record catch of 250,960 tons was made in the 1955-56 season.

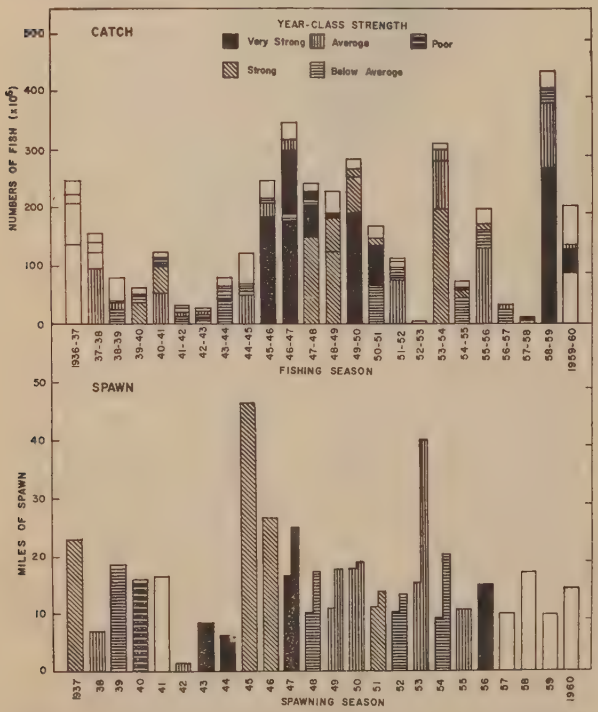

FIGURE 2: Annual catch (in millions of fish) and number of miles of spawn at standard intensity in the lower west coast of Vancouver Island population.

Quotas were first imposed in 1936. The increased exploitation of southern British Columbia stocks in the mid 1930's coincided with a decline in population abundance, the result of the presence of three consecutive poor year-classes. This decrease in abundance aroused fears among fishermen and industry that the stocks were being over-exploited. As a result, catch quotas were imposed on stocks on the east and west coasts of Vancouver Island as an experiment to run for five years. While the results obtained indicated there was little or no justification for continuing the quota system if there was no relationship between the size of the spawning stock and the size of the resulting year-class, sufficient information was not available in 1941 to decide whether or not such a relationship existed. Accordingly, it was decided to continue to quota system and to extend it to other populations. Because of World War II, further study of this problem was not undertaken until 1946-47, when a large scale fishing experiment was proposed. Criginal plans called for the complete closure of the fishery on the west coast of Vancouver Island every third year for 4 cycles or 12 years. The spawning populations would thus be made to fluctuate in a definite and recognizable manner and the dependence, or independence, of recruitment on spawning stock size could be readily determined. Unfort-

unately, the industry did not consider this system economically feasible and a less efficient compromise plan was substituted. Quota restrictions were removed but the closing date for the fishery was retained on the west coast of Vancouver Island. A rigid quota was maintained on the lower east coast to provide a control. Should a significant decrease in catch, spawning population, and recruitment occur over a period of years, it would constitute reasonably satisfactory evidence of the effect of fishing upon the stock.

Results of Research

The following conclusions were drawn from the "west coast" experiment:

1. Catch quotas, between 1936-37 and 1945-46, either for the west coast of Vancouver Island as a whole or for each area individually were not effective in stabilizing the herring catch; in fact, quotas were seldom effective even in limiting catch.

2. The catch in both the upper and lower west coast sub-districts has fluctuated widely, due to variations in the strength of the year-classes contributing the dominant age groups. This is apparent from Figures 2 and 3 which illustrate the relationship between year-class strength, catch and spawn deposition in the upper and lower west coast sub-districts respectively. In the top halves of these figures the height of the bar represents the total catch in numbers of fish. Each bar consists of 4 sections, representing the contributions of the major age-groups; the bottom section shows the number of III-year-old fish in the catch, the second section the number of IV's, the third the number of V's and the fourth or top section the numbers of fish of all other ages combined. Each section is treated to show qualitatively the strength of the contributing year-class (very good, good, average, below average and poor). Catches were large when the III and IV year-olds came from very good or good year-classes, and generally small when fish of these ages came from below average year-classes. In the bottom half, the height of the bar represents the number of miles of spawn, adjusted to a standard intensity, deposited that year. Each bar is gain treated so as to show the relative strength of the year-class that arose from it.

3. There is no relationship detectable in either population between the amount of spawn deposited and the size of the resulting year-class. From the bottom halves of Figures 2 and 3 it can be seen that good year-classes resulted as frequently from small spawnings as from large and poor year-classes as frequently from large spawnings as from small.

4. The variations in year-class strength are, therefore, most probably due to natural factors in the environment and not to variations in the size of the spawning stock occurring as a result of the fishery.

5. It would appear that even with the large increases in fishing efficiency that have occurred since 1946, the removal of quotas did not result in a sufficiently great increase in exploitation to reduce the spawning stock to the point where a direct relationship between spawning stock size and recruitment occurs.

6. It would, therefore, appear that with the degree of exploitation that in practice can now be obtained, catch quotas, at least on the west coast of Vancouver Island, are not required to protect the stock.

While this experiment had provided by 1953-54 fairly convincing evidence that fluctuations in abundance resulted from naturally occurring variation in year-class strength, and furthermore that recruitment was not affected at the levels of fishing intensity likely to prevail following the removal of quotas, it did not provide conclusive evidence on the relationship between spawning stock size and recruitment particularly at the critical low levels of spawning stock size. These findings, while they did not lead to the abandonment of the quota system, resulted in a more liberal policy of quota extensions and a more flexible system of management.

An examination of the relationships between catch, year-class strength and spawn deposition in the remaining populations lead to the conclusion that in all populations the size of the catch was directly related to the sizes of the year-classes contributing the dominant age-groups. Good catches result when strong year-classes are present and poor catches when weak year-classes are. In all populations, with one possible exception, no direct relationship was found between the amount of spawn deposited and the strength of the resulting year-class. The relationship between spawn deposition and the strength of the resulting year-class is shown for each population in Figure 4. In these diagrams, the indices of

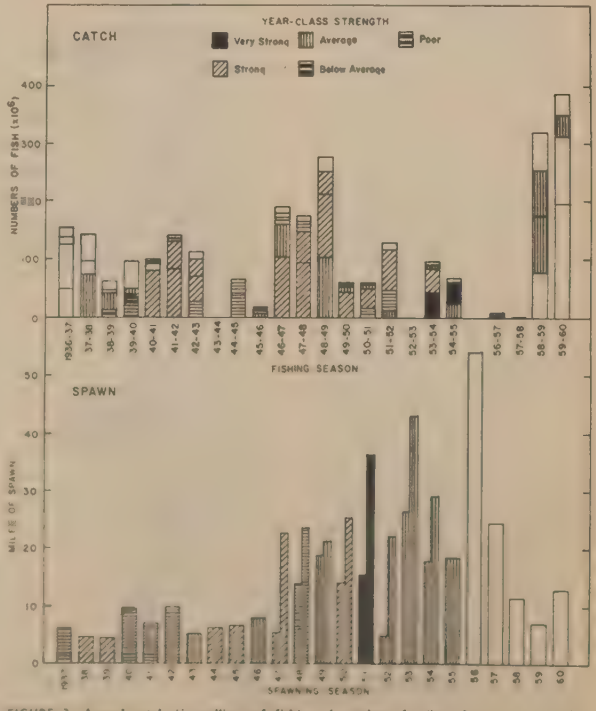

FIGURE 3: Annual catch (in millions of fish) and number of miles of spawn at standard intensity in the upper west coast of Vancouver Island population.

Spectacular increase in efficiency of modern gear has not adversely affected herring populations, and conservationists have none of the problems associated with the salmon and halibut fisheries.

relative year-class strength are arranged in order of size with the largest on the bottom and the smallest on the top. Opposite each index is shown the relative amount of spawn that provided that year-class. The two sides of the diagram will form a triangle if there is a direct relationship between spawn deposition and resulting year-class strength. In herring, estimates of year-class strength can be obtained from data on the size of the catch and on the size of the subsequent spawning population and also from virtual population estimates based on natural mortality rate and numbers of fish at each age in the catch. The two estimates for each year-class correspond closely and have been combined into a single index giving each estimate equal weight. In general, good year-classes have arisen as often from small amounts of spawn as poor year-classes and vice versa.

Conclusions

It would, therefore, appear that in general the British Columbia herring stocks are able to withstand the current high levels of fishing intensity. However, since the seasonal total mortality rate in all populations is in the neighbourhood of 80% or over, no large increases in catch other than those arising from the presence of large year-classes can be expected without possibly endangering the stocks. An examination of models of one population have shown that if total mortality were increased 15% to 95%, a 20% increase in catch might result, but the spawning population would be reduced to an extremely low level. At the present level of fishing intensity the catch in most populations is largely dependent on the strength of the incoming year-class. This year-class contributes somewhere between 50 and 75% of the fish caught. Hence the annual catch fluctuates in response to the strength of this year-class as has already been pointed out. Not only would a further increase in catch (other than those arising from the presence of large year-classes) probably require a more than proportional increase in fishing effort, but the catch would become still more dependent on the strength of the incoming year-class, and hence subject to probably larger fluctuations.

The determination of the relationship between spawn deposition and resulting year-class strength, particularly for small amounts of spawn, remains an important problem in herring management. The maximum sustainable yield will result when the size of the spawning stock left is just in the region where an increase in spawn produced no further increase in the resulting number of recruits. The catch which would allow this escapement would, of course, fluctuate, probably quite widely, depending on the naturally produced variations in the strength of the incoming year-class. To determine the spawn-recruitment relationship, another planned and controlled fisheries experiment would be necessary in which fishing effort is increased and varied to reduce and vary spawning stock size. The changes in the size of the spawning stock would then be associated with estimates of the resulting number of recruits. Such an experiment would be desirable if industry decided a further increase in average catch and fishing effort was worthwhile.

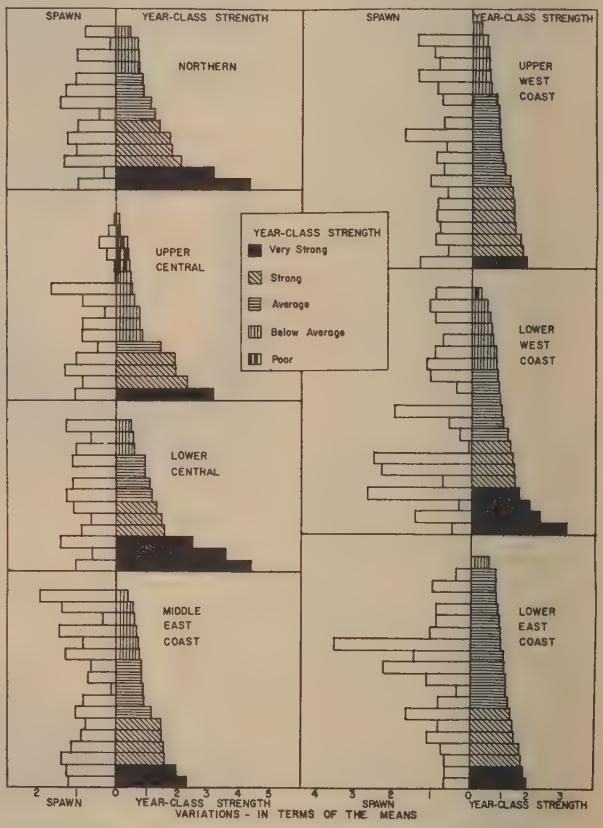

FIGURE 4: The relationship between the indices of year-class strength and of spawn deposition in each population.

The Hecate Strait Model

Hydraulic model provides approach to solution of problems in dynamic oceanography

By NOEL BOSTON,

HECATE STRAIT is one of the richest halibut fishing grounds in the world, and, with the exception of the Bering Sea, is today the most important area adjacent to Western North America for the production of many other species of groundfish. As in many areas, the current systems play an important part in the productivity of the fisheries. An assessment of the physical oceanography of the region would be of great value in the accurate prediction of the availability of fish and fisheries management.

Most species of groundfish produce eggs that float. During this pelagic phase of the early life history the success or failure of a year-brood may be determined by current systems which may carry the eggs out of the region, or return them. For prediction purposes, it is desirable to know what factors in the environment govern the survival of eggs and larvae, and what factors in later life affect the distribution, movements, and concentration of fish on the banks. It is here that physical oceanography can play an important role.

Physical oceanography deals with the distribution (structure) of temperature and salinity, and their resultant density distribution. It also deals with the currents and transport of sea water due to wind, tide, runoff, as well as the motion imposed on the currents and transport due to Coriolis force. It is customary to make observations of the properties of the water from a ship which moves successively from position to position. Such surveys can be considered synoptic, only if the oceanographic state can be assumed to be steady during the period of observations. Below 100 metres depth in the ocean, this is suspected as being as long as one month. However, in shallow coastal seaways, where tidal currents are dominant features, the state is never truly ready. In the study of Alberni Inlet, Dr. J. P. Tully in 1949, solved the tidal and runoff mechanisms by the use of a two fluid hydraulic model. This could not have been done by observations in nature, with the available resources. Since then, several such models of increasing complexity have been built and used to solve oceanographic problems. It has become evident that hydraulic models provide a potent approach to the solution of problems in dynamic oceanography, in those sea regions where the boundary conditions can be defined.

The first step in the construction of a model of the Hecate region was to observe nature. Oceanographic expeditions examined the properties of the water from surface to bottom, at 75 positions (Fig. 1), in the area every two months, through two years (1952-1953). This revealed the major oceanographic features (temperature, salinity, etc.) and their normal seasonal variation, which must be reproduced in the model.

The Author

Noel Boston,

Noel Boston is assistant scientist with the Pacific Oceanographic Group of the Fisheries Research Board of Canada and has been scientist in charge of the Hecate model since 1959.

Born in 1936 in Vancouver Mr. Boston attended Victoria High School and Victoria College before going to the University of British Columbia where he graduated in 1959 with a B.A.Sc. degree in engineering physics. An engineer-in-training he is also a member of the Institute of Radio Engineers.

Reprinted from "The B.C. Professional Engineer" July, 1961

FRB No. 684

In general, the near surface waters are warmed in summer and cooled in winter. There is fresh water runoff from the land which creates a brackish layer over-riding the deep saline water. This fresh water influence is most marked in the inner part of Queen Charlotte Sound and Dixon Entrance, during early winter when the coast rainfall is greatest, and early summer when the large rivers, draining the mountain snow fields, are in freshet. Intruding ocean water, distinct from the seasonally influenced water, lies below. In winter when the ocean winds are from the southwest, the intruding water is from the ocean surface. In summer when the winds are northwest, the intruding water is from the ocean depths, and is relatively cold, and saline. Hence the deep waters, where the flatfish live, are normally warmer in winter than in summer. However, this picture is complicated by the fact that normal winter wind conditions may occur, or even prevail through the summer, depending on the prevailing wind and circulation mechanism. All these conditions must be reproduced.

The tide and tidal currents are the greatest complicating factor in any oceanographic study. In general they move the water in elliptical paths but do not transport it into or out of the region. In order to observe the transport, which determines the environment of the fishery, it is necessary to separate the tidal from the transport components of the currents. It is very difficult to do this by observation in nature. It can be done economically in a model in which the components are known and can be controlled.

On the basis of previous experience with two fluid models, a fixed bed hydraulic model of the Hecate region was designed and constructed. Figure 2 is a photograph of the model before it was enclosed by the roof. The model is 80 feet long, 40 feet wide and has a maximum depth of 4.4 feet. It is designed to provide a first approximation of the dynamics of the region. At present, the model portrays the tidal impulses and effects of the topography of the basin. Later it will be made to show the features of runoff and fresh water influence in the seawater system. Wind effects cannot be demonstrated. However, previous experience has shown that these can be computed once the base state has been established. Further, the model does not show the effect of Coriolis force, which is important in a region of this size. Coriolis force, the deflecting force of the earth's rotation, is often neglected in problems in mechanics because it is negligible compared to other forces. However, the physical forces that maintain the motion of the atmosphere and ocean are of a lesser order of magnitude than the deflecting force and hence it must be considered. Considerable effort is being devoted to determine these corrections, and the mode of application. Finally, it is impossible to preserve geometric and kinematic similarity. Geometric similarity requires equal scaling of the horizontal and vertical dimensions. Kinematic similarity requires that the relative magnitudes of the pressure gradients and local accelerations be maintained. In simulating a region of this size these requirements become incompatible. Since it is the similarity of motions (kinemmatic similarity) that is desired, the model takes on an exaggerated depth form of the protoype. In consequence the model must be carefully compared with the prototype to ascertain the limits of reliability.

The model makes it possible to observe normal and abnormal conditions that may effect the distribution and survival of eggs and larvae. It enables mechanisms to be studied that cannot be studied in nature because the weather cannot be controlled. It provides an indication as to where oceanographic investigations may be most profitable and gives the oceanographer a physical appreciation of the sea's processes. In short, it brings oceanography into the laboratory.

Factors in the Design of the Model

The form of hydaulic models has received considerable attention in the literature of hydraulic engineering. It has been shown that the preliminary criterion of similarity between a model and the prototype is shown first by the Reynolds number and second by the Froude number. Scaling is usually limited to the horizontal and vertical dimensions and velocity, because

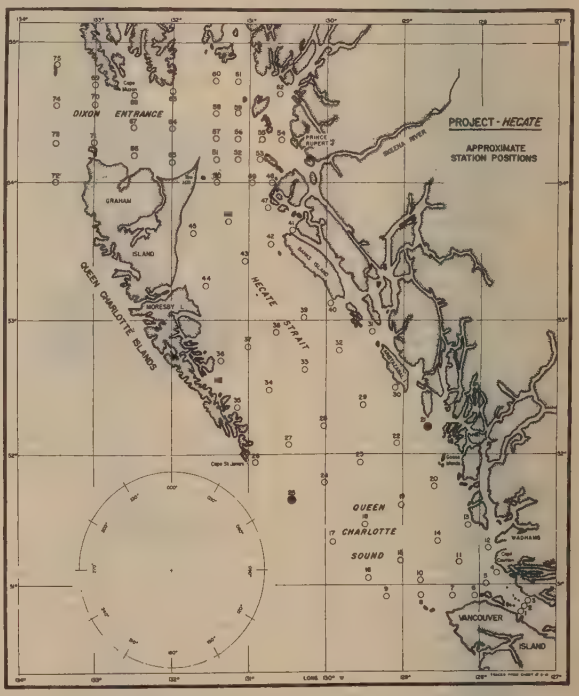

Fig. 1. The Hecate region, showing the positions of observations. These stations were occupied every two months during the years 1952 - 1953.

Table I

DIMENSIONS OF THE HECATE MODEL

	Model	Nature
Maximum length	80 feet	320 nautical miles
Maximum width	40 feet	160 nautical miles
Area	1797 square feet	28,752 square naut. miles
Depth (Max.)	4.4 feet	1320 feet
Volume	50,000 gallons	
Maximum tidal rise	1 inch	25 feet
Maximum tidal volume	150 cubic feet	

SCALE RATIOS

	Model/Nature	
Horizontal	1/24,320	1 foot = 4 naut. miles
Vertical	1/300	1 inch = 25 feet
Distortion	1/81.07	
Time	1/1404.1	61.5 seconds = 1 day (24 hrs.)
Velocity	1/17.321	0.09 feet/sec. = 1 knot

it is impossible to scale gravity, density, viscosity or surface tension. Water is usually working fluid.

The Reynolds number criterion defines the conditions necessary to establish turbulence in the model. The Froude number is the criterion for kinematic similarity. In the Hecate Model, a depth ration of 1:300 (1 inch to 25 feet) was calculated to be adequate to establish turbulence. If the horizontal scale was identical, the model would be more than a mile in length. Since it is water movement, and not the transport of bottom materials, that is being considered it is permissable to distort the scale. To give a sufficient time interval, a horizontal scale of 1:24,320 (1 foot to 4 nautical miles) was chosen. The other model ratios and scales were derived from these decisions in conjunction with the Froude number criterion (Table 1).

The region was modelled on the Lambert orthomorphic conic projection with two standard parallels of latitude. This provides the nearest approach to an equal area projection that can be attained on a flat surface. The distortion does not exceed 1% in any part of the model.

Although the area of interest in the model is the open central portion of the Hecate region, the inflow and outflow from the complex network of inlets and channels along the mainland coast exert considerable influence on the tidal currents in the open central part. However, the model scales are too small for their study. Therefore the approach channels were modelled, but the inlets were simulated by a number of deep, square basins designed to accept the same tidal volume as the inlets which they replaced.

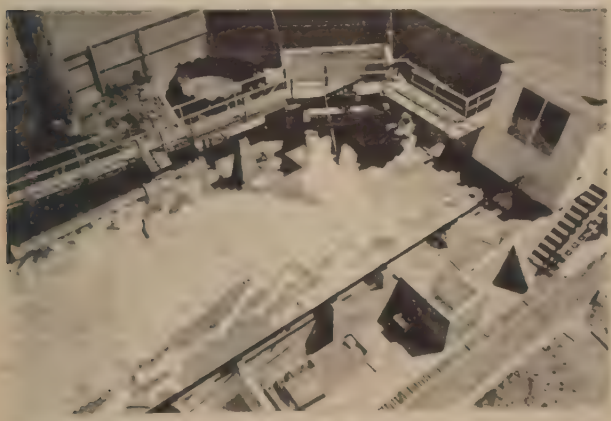

Fig. 2. A photograph of the Hecate Model before it was enclosed by the roof.

Construction

The Hecate Model is located on the heavily forested grounds of the Biological Station at Departure Bay near Nanaimo. An area about 150 feet by 100 feet was cleared of trees and the necessary 105 feet by 65 feet base levelled by bulldozer. This required cutting to a depth of about 10 feet along one edge. The graded area was then covered with two to three feet of gravel in which lines of four-inch drain tile were laid to cope with the subsurface water which appeared during the levelling. A load bearing slab of concrete with one-half inch diameter steel bars at one foot centres in each direction was laid over the gravel to provide the base for the Model. Drain holes at appropriate locations were incorporated in the base along with a water collection system of non-corrosive pipe for draining the Model.

Careful attention was given to levelling when the slab was poured with the result that the greatest differences in elevation over its area were less than one-half inch. The surface of the slab was power trowelled to raise sand to the surface to provide good bonding for walls. This base can be used in the future for other models when the Hecate Model has been removed.

The rough topography and outline of the Model was achieved by stacking standard concrete building blocks to approximate the contours of the region being modelled (Fig. 3). Approximately 9,000 blocks were required. These were locked in place, partly by concrete walls and partly by vertical steel rods and concrete grout in the holes of the outer row of blocks.

The final bottom contours were obtained by grouting to the tops of vertical wires which were set in an initial rough grout coat. The wires were cut to heights by means of a clipper at the lower end of a T-square sliding on a 25-foot horizontal I-beam of a carefully levelled moveable carriage; the I-beam was graduated in nautical miles. The carriage in turn is supported by two parallel, graduated, channel rails, 25 feet apart, which run the length of the model. The rails were adjusted in height by means of two nuts on each anchor bolt in the rail supports. The whole system thus provided a 3-dimensional orthogonal reference system for the model, both for construction and for operation.

The Control and Measurement System

The Model consists of a number of simple components acting together to provide a complex mechanism (Fig. 4). Besides the Model basin, there are the tide computer, the tide generators, the tide gauges, the recorder, and the current observing system.

The Tide Computer

The tide-producing forces are due to the sun and moon. The rise and fall of the tide in any locality can be expressed mathematically by the sum of a series of harmonic terms having constant relations to the forces of these two bodies. Therefore the height of the tide (H_i) at any time and place is described by an equation of the form:

$$H_i = H_o + \Sigma A_i \cos(w_i t + \theta_i)$$

where H_o is mean sea level, A_i is the amplitude of the ith harmonic term, or component, w_i is the rate of rotation of the ith component and θ_i is the phase of the ith component at time $t = 0$. The tide computer is a mechanical-electrical analogue of this tidal equation.

To account for all the semi-diurnal, diurnal, seasonal, and yearly effects of the sun and moon, more than thirty terms are required. However, in the Hecate region, seven tidal constituents are sufficient to account for 96% of the tidal rise. It is not necessary that the model produce the exact tidal heights observed in nature. Rather it is required to produce representative sequences through periods of three months to a year. Hence it is only necessary to reproduce the effects of these seven components. The difference between the predicted tide in the Model and that predicted in the tide tables will never be more than 0.02 inches (½ foot in nature) or more than one second (23.4 minutes in nature) at any time. This is within the limits of variation of the natural tides. This does not imply that a similar error in predicting the tide in the model or measuring its rise in the model can be accepted. The tide, as predicted by the computer, must be accurately reproduced in the model.

The tidal equation less mean sea level (H_o) is represented by a train of gears, each rotating at speeds relative to the moon's semi-diurnal component. It is driven by a synchronous motor at a speed adjusted to the time scale of the model. One rotation occurs in 31.841 seconds which in model times is equivalent to 12 hours, 25 minutes, 14 seconds of natural time. The speed (w_i) of the other gears is then fixed.

Fig. 3. The rough contours. The two plates form the tide generation system. The nearer plate is across Queen Charlotte Sound. The rails mark the reference co-ordinates for applying the final contours.

Whereas the relative speeds of the components are the same everywhere, the amplitudes (A_i) are different for each position in the sea. The amplitude of each component is established electrically. On the end of the axle of each gear, there is a sine-cosine potentiometer. The amplitude is determined by a constant input voltage, which is specific for each component. Since there are potentiometers on the end of each of the axles of each gear, two tides can be computed independently. Because the constant input voltage can be adjusted to suit any local conditions, the machine can compute the tides for any two places in the world simultaneously.

As the gears rotate, sinusoidal voltages are produced and summed. The current feeding the drive motor varies according to the output voltages of the tide-computer. Thus the position of the tide generator depends on the output of the computer. Each generator in turn operates a pulley and potentiometer arrangement. A voltage is applied across the potentiometer and its output is a continuous indication of generator position. This signal is returned and compared with the computed position. The error signal is amplified and applied as a correction to the motor (Fig. 4). This feedback system allows each generator to follow the computed tide within 0.1%.

The Tide Generators

The problem of introducing tides into the model was difficult because of the volume of water involved and the accuracy with which it must be moved. On the maximum flood tides, about 100 cubic feet of water must be introduced into Queen Charlotte Sound in 16 seconds, and about 40 cubic feet of water into Dixon Entrance in the same time. These same volumes must be discharged in similar periods. This requires a maximum flow of about 10 cfs which is not available on the model site. Hence the water has to be stored and recirculated.

Conventional tide generating mechisms were considered and rejected on the grounds of excessive mass (inertia) and power, either in counter weights or pumps. Instead, a novel mechanism was designed which produced the desired flow with the necessary accuracy and required for power only two ¼ hp. motors! As shown diagrammatically in Fig. 5, a water-tight vertical plate hinged along the bottom edge, is rocked to and fro (flood and ebb) between two basins by a motor drive screw. Sealed behind each plate is a rectangular basin full of water to counter balance the pressure forces on the plate. As the plates lean toward the model, the tide rises in the model and falls in the rectangular basins The head difference across each plate counter balances the moment of the plate about its hinge. However, the pressure difference is small (¼ lb. per square inch) so that a very simple seal is required. Sponge fitted along the edges of the plates slides on sheets of stainless steel

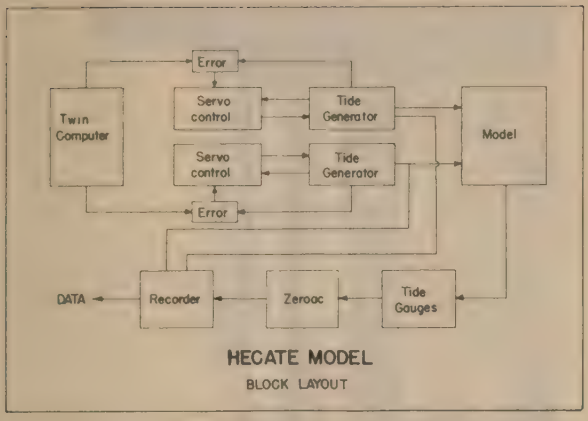

Fig. 4. The Hecate Model control and measurement system.

fitted into the model form. The moment of each plate can be adjusted (by adjusting its weight) to equal the moment caused by the restoring force due to the head difference between the basins.

The range and speed of movement is controlled by the tide computer. One plate, 33.6 feet long, is across the approach to Queen Charlotte Sound, and another plate, 9.4 feet long, is across Dixon Entrance (Fig. 3).

The Tide Gauges

The tide gauge is a servo-mechanism which translates the vertical movement of the water surface into voltage. A two-pole motor, by means of a nut and screw arrangement, operates a moveable sensing probe carrying two long and one short sensing needles. The long, outer needles carry an electrical potential between them, while the short centre needle serves as the sensing electrode. When the short needle is not in contact with the water, the probe is driven downwards. As soon as it comes in contact with the water, the potential is detected by the short needle due to the conductivity of the water. This causes a fast-switching relay to reverse the polarity of the motor and the probe is driven upwards. As soon as contact with the water is broken, the downward movement recommences. The screw is connected by means of a belt and pulley to a potentiometer across which a voltage is applied. The change in potential is linearly proportional to the vertical movement of the probe, and hence the water surface.

The surface tension of the water causes the surface to be pushed down about 1/64 of an inch before being punctured, and to cling to the needle for the same distance when being withdrawn. To nullify this effect (which occurs over a distance representing six inches in nature), the centre sensing needle is attached to the diaphragm of a small radio speaker oscillating on line voltage at 60 cycles per second with an amplitude of 1/64 of an inch. This shakes off the film and allows the probe to follow the true surface of the water to within 0.01 inches and periods as fast as one-half second.

The entire tide gauge, electronics, pulley arrangement, and motor is contained in a package occupying one cubic foot and weighs less than twenty pounds. They are portable and may be set wherever a sufficient footing exists. To set them at the same elevation would require elaborate lavelling procedures. Therefore an electronic balance is employed to calibrate the signals from each gauge (the Hecate Model has six) so that the same voltage is recorded from each gauge at mean sea level, which provides an easily attained common reference. Deviations (tidal rise) may be discerned within 0.02 inches (one-half foot in nature) which is adequate to measure the tidal slope of the sea surface.

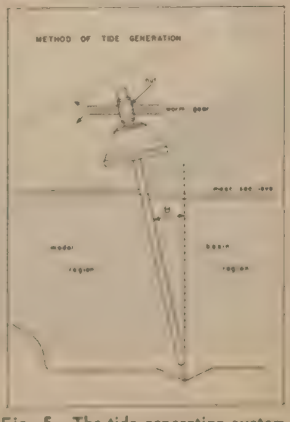

Fig. 5. The tide generating system.

The Recorder and Current Observing System

The predicted tides, the tide generator movements, and the tidal heights in the model, are registered on an eight channel Sanborn recorder. In this instrument a strip of heat sensitive graph paper moves at a constant speed under a roller. The voltages from the several units actuate D'Arsonval type galvonometers which cause hot wire styli to move over the paper, making a record of voltage as a function of time. The chart scale translate these voltages into the desired terms of height. This is the only part of the physical model and electronic system that was not designed and built on the site.

Since the current systems are complex, varying with position, depth, and time, visual observations are inadequate. There must be a mechanical system of comprehensive observation and memory.

Floats or dyes are introduced into the model at desired positions. The water movement is determined by photographically observing the successive positions of the dye patches or floats, at successive high tides. Thus, the semi-daily ebb and flow of the tides are ignored, and only the net progress through successive tide cycles is considered.

The camera is located 90 feet above the model on a platform attached to a 110 foot spar tree which has been barked, preserved, and guyed. A service car, operated by a winch, carries an operator up to the platform which allows a view of the model from a height equivalent to 360 miles in nature. The operator installs the camera and associated equipment and returns to the ground. The camera has a 50 foot magazine for 35 mm colour film. A wide angle lens is necessary to view the entire model in a single frame. The film is driven by a motor which is triggered by the computer. When a photograph has been taken, the motor automatically reels the film into

position and cocks the camera for the next exposure. To synchronize the photographs with the chart recording, a clock which show days and hours in nature is mounted along side the model, and appears in the photographs (Fig. 2, left center). The triggering of the camera is also shown on the recorder thus allowing exact correlation between the photographs and tide records.

Photography is carried out at night to defeat temperature gradients caused by solar heating. As many as five density stratified layers have been observed due to this effect. Night photography requires two strobolights, which give 800,000 candle power in a five-hundredth of a second flash. They may be operated as frequently as every ten seconds.

Initially, the presence of a camera 90 feet above the model precluded the possibility of having the model enclosed. However, since photography can be carried out only when the model has been extensively tested, and in fact, occupies only a small fraction of the operating time, a roof was constructed with two sliding portions 35 feet by 30 feet which can be moved horizontally to expose the model (Fig. 6). The roof allows calibration and cotidal testing to be protected throughout the winter. Photography is done during still summer nights.

For study, the photographs are projected onto a map of the region. The successive positions and direction of movements are manually transferred onto a single map, where the circulation patterns are collated and analyzed.

Conclusion

This model has brought oceanography into the laboratory on a scale never before attempted. It is possible to observe, and re-observe normal and abnormal conditions, to study particular features and mechanisms, such as the dissipation of fresh water in the sea, the effects of density stratification on the current system, etc. None of these can be adequately studied in Nature because the weather cannot be controlled. The model provides an indication as to where oceanographic investigations may be most profitable and gives the oceanographer a physical appreciation of the oceonographic processes in nature.

Acknowledgements

I wish to give credit to Mr. L. D. B. Terhune, who was responsible for the construction of the physical form of the Hecate Model, and to Mr. Marcel Pirart, who was responsible for its instrumentation. This report is, in part, a description of the excellent work done by these men.

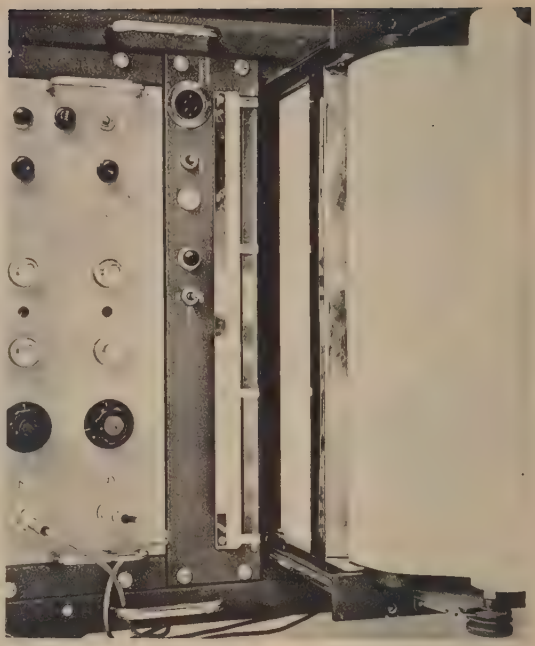

Fig. 6. The recorder. Tidal traces show on channels 1, 2, and 4. Channels 7 and 8 monitor the position of the tide generators.

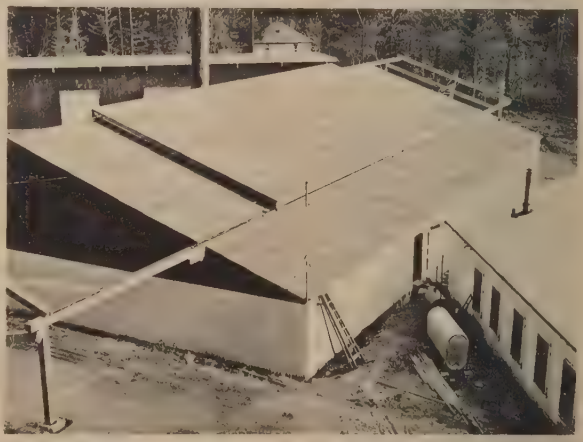

Fig. 7. The roof in closed position. Central raised section consists of two equal panels mounted on casters. The casters run along channel iron. In open position, panels are over outrigger beams, shown at either end of the roof.

Moisture in Fish Blocks Processed from Very Fresh Fish

by W. J. DYER, and DORIS I. FRASER

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(Reprinted from CANADIAN FISHERMAN, August 1961)

FRB No. 685

Moisture in Fish Blocks Processed from Very Fresh Fish

"Ice pockets" or messiness in a box of fish can be prevented by careful handling of the fish and watching certain temperature factors. Here are some suggestions for plant managers.

by W. J. DYER, and DORIS I. FRASER
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MOST OF THE DIFFICULTIES associated with "wet" fish blocks used for the preparation of fish sticks arise from the drip which accumulates in the trays and is frozen into the blocks. This can be minimized by routing the trays into the freezer immediately after packing, so that draining and accumulation of drip in the tray do not have time to occur. This is discussed in a previous article (W. J. Dyer, "Wet" Frozen Fish Blocks, Department of Fisheries *Trade News*, Nov. 1960, p. 8-9).

The moisture arises from two sources: (a) that left on the fillets after washing, dipping in preservative or antibiotic solutions, or from the fish lying in puddles of juice on the packing tables; (b) that leaking from inside the fish itself. Normally, the latter is small but in the case of fish processed while it is very fresh, prior to the passing of rigor, it may become large.

In some fish plants processing shore fish, for instance Newfoundland trap cod, this problem can be very troublesome. However, we also know that fish in the very fresh pre-rigor state are being processed in many plants in many countries as well as aboard factory ships, and that the best frozen fish are produced in this manner.

There must therefore be a reason why the pre-rigor fish can be processed successfully in some cases and not in others. To understand what is happening, let us take a look at some of the changes, both physical and chemical, which take place in the fish muscle immediately after death, as it goes into and through "rigor mortis."

What are these changes?

Most obvious are the physical ones. Soon after death the previously flexible muscles become stiff, hard, and sometimes contracted, i.e., rigor has set in. Eventually the muscles slowly relax again and rigor has been resolved. Previous to rigor, the surface of the cut muscle is dry, but as rigor proceeds the surface becomes wet and under some conditions large amounts of "weep" or drip may exude. These changes are the result of the biochemical and enzymic processes continuing in the muscle after death. The sugar energy reserve compounds, in this case glycogen (a starch-like compound), are converted to lactic acid, which increases the acidity of the muscle tissue. High-energy phosphate compounds, which normally supply the energy for muscular contraction, are also hydrolyzed and allow the muscle proteins to contract and stiffen, that is, develop "rigor mortis."

The speed and the extent of these reactions control the time of onset and the duration of rigor and they depend on several factors, particularly the energy reserves in the muscle at the time of death, and on the temperature. When the pH or acidity of the muscle is changed by the lactic acid formation, the moisture-binding capacity of the protein of the flesh is reduced and drip formation may result.

Curiously, almost no published data exist on the speed of these changes in most of our commercial fish species. We have measured the rate of several of these chemical processes at 32°F., 50°F., and 75°F., and found that they were about one-half completed in 12 hours, 6 hours, and 1 hour respectively, when rested, freshly killed cod from a tank were used. Temperature then is a very important factor. In these experiments it was noted that the fillets at 75°F. underwent marked shortening, although only small amounts of drip developed.

Fish Plant Conditions

Further experiments were set up to determine the time of onset of rigor, shortening and drip formation at various temperatures which might be encountered in a fish plant.

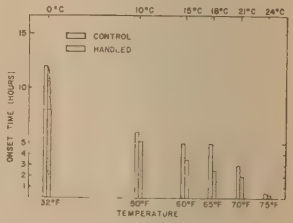

Figure 1. — Temperature effect on the time to onset of rigor in quickly killed cod, filleted immediately. The cod were held in a tank at 36°F. and the fillets, contained in polyethylene bags, quickly brought to the required temperature in water. (For explanation of "handled," see text).

In Figure I, the time of onset of rigor in hours is indicated at various temperatures from 32 to 75°F. It varies from about 12 hours at ice temperature to only ½ hour at 75°F. These data are for rested

cod (not feeding) held in a tank of seawater at about 36°F. The fish were killed without struggling, immediately filleted and the fillets, enclosed in a polyethylene bag, brought to the required temperature by immersion in water. Resolution of rigor did not occur until 2 to 3 days at 32°F. and about 6 hours at 75°F. In exhausted fish, as would usually be obtained under commercial conditions, the reserves of energy-yielding compounds would be much less and the time to onset of rigor and its duration would be somewhat shorter.

The amount of shortening and the loss of drip were also measured in these fillets and the results are shown in Figures 2 and 3.

As before, no shortening was observed up to 60°F. but marked contraction occurred above this temperature reaching 33% at 75° (after 6 hours). These contracted fillets became very rough and the muscle fibres became wavy and crimped in appearance as illustrated in Figure 4. Two fillets taken from the same fish are shown. One was stored in ice, the other was brought to room temperature. The photograph was taken about 30 minutes after filleting.

Accompanying the shortening and rigor was the production of varying amounts of drip, a result of the loss of water-holding capacity of the protein of the muscle. The drip formation closely paralleled the shortening. No drip appeared below 60°F. but about 5% was formed at 70 to 75°F. This was much less than had been observed in the plants where trouble with excess drip was being experienced. Accordingly, some fillets were "handled" as in these plants by slapping them down on a table about ten times. The results obtained are shown in the cross-hatched columns of Figures 1 and 3 and the dotted line in Figure 2. Time to onset of rigor was decreased to about two-thirds of the unhandled fish, and some shortening occurred at 50 to 60°F. It increased very slightly at temperatures up to 70°F. but drastically increased to about 12% at 75°F. The latter condition was similar to the conditions observed in the plants where excessive drip formation occurred in the blocks as they were packed awaiting transfer to the freezer. It appears that a combination of high temperatures and rough handling results in the greatly increased drip formation.

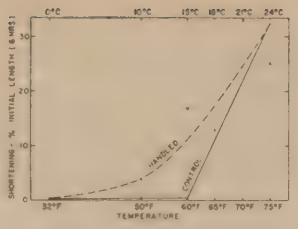

Figure 2. — Shortening of cod fillets as affected by temperature and rough handling

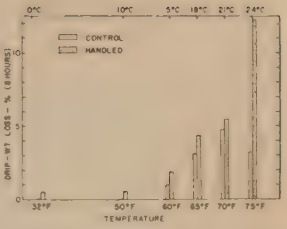

Figure 3. — Drip in cod fillets as related to temperature and rough handling. Drip was the weight loss by free drainage in 8 hours, evaporation being prevented by enclosing the fillets in a polyethylene bag.

Plant Manager's Problem

The critical factor, of course, from the plant manager's viewpoint, is the rate of formation of this excessive drip. If it occurs slowly over a period of several hours, he will have time to get the fish frozen before objectionable amounts of drip have accumulated in the packages or blocks. However, if it is formed rapidly, his problem in getting the blocks into the freezer rapidly enough may become acute, and perhaps practically insurmountable. The rate of shortening at the various temperatures is shown in Figure 5.

It is seen that most of the shortening (and drip formation) occurs in the first 2½ hours at 65 to 75°F. However, in the handled fish at 75°F., it takes place in the first half hour. This, of course, is just the stage where the filleted fish are lying on the packing table awaiting packing, or are in the packages awaiting to go into the freezer. Not only is there a messiness, because of draining out of the packages and freezing of juice on the outside of the blocks in the freezer, and a loss in broken fish sticks in fish processed under these conditions, but there is also a loss of product weight of 10 - 12%, a not inconsiderable economic loss when figured on a season's production.

These results explain the reason

Figure 4. — Photograph of two fillets taken from the same fish. One was held 30 minutes at 32°F., the other, which shows contraction, was stored the same length of time at 75°F.

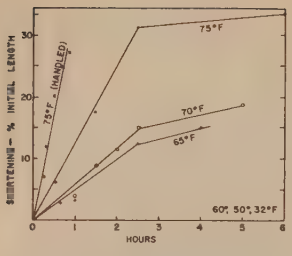

Figure 5. — Rate of shortening of cod fillets at various temperatures.

for the trouble experienced in some plants packing pre-rigor cod into fish blocks. But it was the poor conditions of processing, allowing the temperature to rise, along with excessively rough handling, which led to the troubles; not the use of pre-rigor fish.

It is planned to extend these experiments using exhausted fish and also feeding fish to determine the effect of the use of very fresh fish on the freezing and thawing characteristics of frozen packs.

The present results, carried out with freshly killed rested fish from a tank, show that if the temperature of the fillets is allowed to rise above 50-60°F. there will be some shortening and drip formation. If the fish and the fillets are roughly handled as well, the drip formation is very much increased. This may reach 10 - 12% at high temperatures. In such a case the drip was formed mainly in the first half hour coinciding with the period during which the fillets would be in the packages waiting to go into the freezer. It is not surprising that the release of 10 - 12% of water or juice in a box of fish leads to the formation of "ice pockets" and of messiness.

Thus for processing of very fresh fish, the fish and fillets should not be allowed to warm up above 50 - 60°F. and they should be handled carefully.

Observasjon om vinteren av en knølhval som dier sin unge på Grand Bank, Newfoundland

Winter Sighting of a Humpback Suckling its Calf on the Grand Bank of Newfoundland

Av (by) Gordon R. Williamson

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«Knølhval: 8. mars 1961, 50°50'V 42°53'N, over den sydlige spissen av Grand Bank. Dybde 80 favner, havets overflatetemperatur 1,7°C. Blåst observert ca. 300 m rett forut. Fikk å skue en stor hvit sveiv som viftet forsiktig i luften. Den var hvit på begge sider og bulet langs kantene. Med vår laveste fart på ca. 3 knop styrte vi mot hvalen. Den fortsatte med å ligge på siden og vifte med sveiven, som var svær. Da fikk vi øye på en liten hval like ved siden av morens mage. Vi var øyensynlig vitne til at ungen ble diet. Et fantastisk syn! Vi passerte mindre enn ti meter fra de to, men de røbet ikke med en bevegelse at de hadde sett oss. Ca. 200 meter unna så vi en annen voksen knølhval».

I dette utdrag fra den dagboksnedtegnelse jeg gjorde ombord i vår forsøkstråler «A. T. Cameron», finner man de viktigste detaljene vedrørende denne høyst interessante observasjon. Det var ikke bare det at vi på bemerkelsesverdig kort hold så en knølhvalmor som diet ungen sin, men vi var usedvanlig

«Humpback whales: 8th March 1961, 50°50'W 42°53'N, over the southern tip of the Grand Bank. Depth of sea 80 fathoms, surface temperature of sea 1.7°C. Blowing sighted 300 yards dead ahead. Lo and behold a great white flipper gently waving in the air. White both sides and knobbed along the edges. At minimum speed of about 3 knots we steered for the whale. It continued to lie on its side and wave its flipper — a vast thing. Then we noticed a small whale just beside mama's tummy. Obviously we were witnessing suckling. A fantastic sight! We passed within ten yards of the pair but not a move did they make to acknowledge our presence. About 200 yards away another adult humpback was seen.»

This extract from the log I wrote aboard our research trawler «A. T. Cameron» contains the main details of our most interesting observation. Not only did we have a remarkably close view of a mother humpback suckling her calf, but we were unusually lucky to see a humpback at all on the

Table 1. Summary of observations in Newfoundland area.

Observation		Date	Place		Observer
Species	Number		Position	Locality	
Large dolphin, probably pilot whale **Globicephala melaena**	1	2 March 1961	44°30'N 52°30'W	SW edge of Grand Bank	author
Fin whales	2 adults	7 March 1961	43 17'N 51°38'W	SW edge of Grand Bank	author
Humpback whales	2 adults 1 calf	8 March 1961	42°53'N 50°50'W	Southern tip of Grand Bank	author
Fin whales	1 adult 1 calf	ditto	ditto	ditto	author
Dolphins about 10 feet long, with remarkably white flanks. Probably **Lagenorhynchus acutus** or **L. albirostris**	6	ditto	ditto	ditto	author
Humpback whales	2 adults	April 1946	47°50'N 52°45'W	close to Cape St. Francis, Newfoundland	Captain Borgen
Humpback whales	several adults	April 1960	45°20'N 61°00'W	off Mulgrave, Nova Scotia	Captain Borgen
Rorqual whales	«spouting all around us»	December 1960	47°N 45°W	on Flemish Cap bank	Factory trawler «Bertolt Brecht» of Rostock

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FRB No. 686

heldige som i det hele tatt så en knølhval på Grand Bank på den tiden av året. Litt diskusjon omkring denne observasjon er neppe uberettiget.

Sjøen var ganske krapp, men solen skinte klart, og det så ut som om hvalen diet mer eller mindre uavbrutt i de åtte minuttene vi så den. Vi så bare den blåste to ganger. Blåstet gikk da rett opp, og ut fra dette så det ut til at den rullet seg i vanlig stilling for å puste. Resten av tiden lå den på siden

Grand Bank at this time of year. Some discussion of the sighting may not be out of place.

The sea was quite choppy but the sun was shining bright, and for the eight minutes the mother was in sight she appeared to be suckling more or less continuously, only being seen to blow twice. The spout was quite vertical on these occasions, thus it would appear that she rolled completely

med sveiven høyt opp i luften. Sveiven som var helt hvit både på over- og undersiden, svinget forsiktig fra side til side over ca. 60 grader, og det var interessant å se hvor bøyelig den var. Man kunne se at den bøyde seg betraktelig, og da den sveivet rundt, så man av og til oversiden (som på skissen) og av og til undersiden. Den venstre spiss av halefinnen var av og til synlig over havflaten som om halen kanskje beveget seg forsiktig fra den ene siden til den andre på samme måte som sveiven. Hvalen ble imidlertid ikke drevet fremover.

Ungens blåst var for lite til at vi kunne se

upright to breathe. During the rest of the time she lay on her side with her flipper held high in the air. The flipper, of which the upper surface as well as the under surface was almost completely white, was gently moved from side to side through about 60 degrees and it was interesting to see how flexible it was. It could be seen to bend considerably, whilst rotation at the base caused sometimes the upper surface (as shown in the sketch) and sometimes the under surface to be visible to the onlookers. The tip of the left fluke occasionally cut the surface of the water as if, perhaps, the tail was being moved gently from side to side in the same manner as the flipper. No locomotion was resulting however.

The spout of the calf was too small to be no-

det, og selve ungen så vi bare da vi passerte den. I den krappe sjøen var det vanskelig å avgjøre nøyaktig hvor lang den var, men den var ikke mer enn halvparten så lang som moren. To ganger observerte man at ungen kom opp for å puste, og begge gangene sto den helt oppreist.

På Grand Bank er mars-måned midt på vinteren, og pakkisen i Labrador-strømmen er ikke mer enn 200 sjømil nordenfor det stedet vi observerte hvalene. Selv om de fleste knølhvalene i den vestlige delen av Nord-Atlanteren pleier å være i de varme farvannene lengre syd (sannsynligvis i Det karibiske hav) på denne tiden av året, så vet man at Nordishavet og Sydishavet ikke er helt tomme for hval om vinteren. Vi observerte tydeligvis noe som var sjeldent. Det var imidlertid usannsynlig at ungen var født i slike kalde farvann, og den var så stor at man ikke kan utelukke at den var født et annet sted på den tid av året som er normal for fødsler, en gang mellom november[5] og februar.

I denne forbindelse er det verdt å legge merke til at på vestsiden av Nord-Atlanteren trenger ikke en hval vandre så langt for å komme fra de kalde ernæringsfeltene til meget varme farvann. Bare 200

ticed and the calf itself was only seen as we actually passed it. Its length in the choppy sea could not be estimated accurately but it was not more than half as long as its mother. The calf was twice seen surfacing to breathe and was completely upright both times.

March on the Grand Bank is a mid-winter month with the pack ice of the Labrador current lying less than 200 miles to the north of the spot at

Mother Humpback suckling her calf
In this sketch the calf is shown just as it surfaced to breathe

which the whales were seen. Although the majority of the humpbacks of the western North Atlantic would be in warm southerly waters (probably in the Caribbean area) at this time of year it is known that the winter Arctic, and Antarctic, seas are never entirely deserted by whales. We manifestly observed a pod of the minority. It was unlikely that the calf was born in such cold waters, however, and its size would not preclude it having been born elsewhere at the normal season, somewhere between November[5] and February.

In this connection it is notable that on the western side of the North Atlantic a whale does not have to migrate far to get from its cold feeding grounds to very warm waters. Only 200 miles to the

sjømil sydøst for Grand Bank finner man havtemperatur på 15°C og høyere — selv i mars. Golfstrømmen stryker med stor kraft opp fra syd, og den høye temperaturen i strømvannet gjør at de nordligste koralrev på den nordlige halvkule trives ved Bermuda på 32°N. Det kan godt være at slike farvann oppfyller behovet til knølhvalen i forplantningstiden.

Avstanden mellom ernæringsfeltene og formeringsfeltene kan således være bare ca. 800 sjømil for enkelte knølhval i den vestlige delen av Nord-Atlanteren — en forfriskende svømmetur på litt mer enn en uke hvis hvalene holder en svømmehastighet på 4,3 knop, som man har observert andre steder.[3]

Det er i juni, juli og august at skippere på fiskebåter rapporterer store forekomster av knølhval og hval av finnhvalartene fra den sydøstlige grunnen av Grand Bank. Her er det ca. 30 favner dypt, mens det ellers på Grand Bank er 40—60 favner, og det er et område som er meget rikt på føde i disse månedene. Uhyre mengder av lodde, *Malotus villosus*, samler seg der for å gyte, mens man samtidig finner like store mengder av ung kolje, *Melanogrammus aeglefinus*, som ernærer seg av lodderognen.[9]

Som det fremgår av kartet, stikker østkysten av Newfoundland ut i havet, slik at alle hvalene i den vestlige delen av Nord-Atlanteren må passere dette område under vandringen nordover og sydover. Ved siden av at Grand Bank er så rik på føde, forklarer dette i stor utstrekning hvorfor man kan observere så mange hval der. (Det er noen få hval som vandrer vest for Newfoundland gjennom stredet ved Belle Isle.)

Det er hyggelig å berette at de store artene i dette område i den siste tiårsperiode i betraktelig grad har vært forskånet for fangst, idet det bare i liten utstrekning er blitt drevet fangst på dem fra de landstasjonene som er vist på kartet. Den lille Touqussaq-stasjonen i Davis-stredet på Vest-Grønland ble nedlagt efter 1958, og Dildo fanger bare tilfeldige store hval, idet den hovedsakelig fanger grindhval og minkehval. Efter brannen i 1959 begynner Hawke Harbour fangsten igjen i sommer med én hvalbåt. Hvalur H.F. på Island driver den største stasjonen med en årsfangst på ca. 400 hval, men den ligger langt mot øst, og det kan godt være at den ikke fanger hval av bestanden ved Newfoundland. Dessuten er blå- og knølhval beskyttet i området under Den internasjonale hvalfangstkonvensjon. Beskyttelsen under Konvensjonen utløper i 1964 for knølhval og i 1965 for blåhval.

Under syvdagers-toktet med «A. T. Cameron» til den sydlige delen av Grand Bank, så vi også flere andre hval og småhval (tabell 1) til tross for at været var meget dårlig. Det er verdt å nevne at vi

south-east of the Grand Bank water temperatures of 15°C and higher are to be found — even in March. The Gulf Stream sweeps powerfully up from the south and its high temperature allows the most northerly coral reefs of the hemisphere to flourish at Bermuda in 32°N. It may well be that such waters fulfil the requirements of breeding humpback whales.

The distance between feeding and breeding grounds would thus be only about 800 miles for some humpback whales in the western North Atlantic — an invigorating swim of little more than a week's duration if the migrating speed of 4.3 knots observed elsewhere were maintained.[3]

It is in June, July, and August that fishing skippers report large numbers of humpbacks and rorquals from the south-east shoal of the Grand Bank. The water here is about 30 fathoms deep, compared with 40—60 over the rest of the Grand Bank and it is an area very rich in food during these months. Vast numbers of capelin, *Mallotus villosus*, congregate to spawn while equally vast numbers of young haddock, *Melanogrammus aeglefinus*, are also present feeding on the capelin eggs.[9]

The east coast of Newfoundland, as can be seen from the map, is the great headland around which all whales of the western North Atlantic must pass on their north-south migrations. This fact, in conjunction with the richness of the Grand Bank as a feeding area, does much to explain the abundance of whales that are observed there. (There are a small number of whales that migrate west of Newfoundland through the Strait of Belle Isle.)

It is pleasant to record that in the last decade the large species of the area have enjoyed considerable freedom from whalers, being hunted on a small scale only, from the shore stations shown on the map. The small Touqussaq station on the Davis Strait in West Greenland closed down after 1958, and Dildo catches only occasional large whales, being primarily a Pilot and Minke whale station. Hawke Harbour, after being burnt down in 1959, is still re-opening with one catcher this summer. Hvalur closed H.F. in Iceland operates the largest station, with an annual catch of some 400 whales, but it is far to the east and may well not tax Newfoundland whale stocks. In addition, blue and humpback whales are protected in the area under the International Whaling Convention. The period of protection expires in 1964 for humpbacks and 1965 for blue whales.

On the «A. T. Cameron»s seven-day cruise on the southern Grand Bank we also saw several other whales and small cetacea (Table 1) despite very rough weather. Notable was a fin whale and calf

så en finnhval og en unge som passerte oss bare fem minutter efterat vi hadde sett knølhvalene. Da vi kom tilbake til St. John's, satte jeg meg i forbindelse med kaptein Johan Borgen som eier stasjonen i Hawke Harbour og som har høstet erfaring som hvalskytter verden over, for å spørre hva han visste om knølhval i farvannene ved Newfoundland. De opplysningene jeg fikk av ham, er gjengitt nedenfor, og jeg vil gjerne få takke ham for hans velvillige assistanse og for den interesse han viste.

«Man finner knølhval utenfor den nordlige delen av Newfoundland om sommeren og høsten, men det er ikke mange av dem. Man kan se flest dyr i tiden fra juli til september. Ofte kan man se mødre med unger. Andre steder i verden har jeg ofte sett knølhval som dier sine unger. Vanligvis ligger moren på siden, men jeg har noen ganger sett at hun svinger med sveiven i luften, eller at hun slår i vannet med den.»

Fangstsesongen varer her fra juni til november, og nesten alle de hvalene som er fanget ved Hawke Harbour og Dildo, har spist lodde, som kommer inn til kysten i uhyre mengder om sommeren for å gyte. Enkelte hval har spist lanternefisk, av familien *Myctophidae,* som lever på dypet om dagen, men som kommer helt opp i havflaten om natten. Krill, *Thyssanoessa raschii, T. inermis,* og *Meganyctiphanes norvegica,* som finnes i ganske store mengder ved Newfoundland, selv om det kvantitativt er mindre forekomster av krill her enn i Sydishavet, blir hovedsakelig spist av blåhval tidlig og sent i sesongen, før og efterat lodden kommer.

Jeg vil gjerne få takke Dr. David Sergeant for de antydede identifiseringer av de små hvalene, mannskapet på «A. T. Cameron» for deres arbeidsiver på toktet, Ned Rowe for hjelpen med å lage kart-illustrasjonen og Robin Molson i fiskeridepartementet for adgang til kontrollørenes rapporter vedrørende maveinnholdet hos hval.

that passed only five minutes after we had seen the humpbacks. On our return to St. John's I contacted Captain Johan Borgen, owner of Hawke Harbour Station and gunner of world wide experience, to ask what he knew of humpbacks in Newfoundland waters. The information he gave me is noted below and I take pleasure in thanking him now for his kind assistance and interest.

«Humpbacks are to be found off the north of Newfoundland during the summer and autumn but they are not numerous. The majority are seen during July to September. Mothers with calf are sometimes seen. In other parts of the world I have often seen humpbacks suckling their calves. Usually the mother just lies on her side but I have sometimes seen her waving her flipper about in the air or slapping the water with it.»

The whaling season here extends from June to November and nearly all whales caught at Hawke Harbour and Dildo have been eating capelin, which come inshore in vast numbers to spawn during the summer. Occasional whales have been eating lantern fish, family *Myctophidae,* which, though living in the depths by day, come right up to the surface at night. Krill, *Thysanoessa raschii, T. inermis,* and *Meganyctiphanes norvegica,* which is quite abundant in Newfoundland waters though not in Antarctic quantities, is mainly eaten early and late in the season before and after the capelin congregate, and by blue whales. .

I would like to thank Dr. David Sergeant for the suggested identifications of the small cetaceans, the crew of the «A. T. Cameron» for their willing work on the cruise, Mr. Ned Rowe for help in preparing the map illustration, and Mr. Robin Molson of the Department of Fisheries for access to the inspectors' reports referring to the whale stomach contents.

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Pilot Plant Unit for the Continuous Methanolysis of Glyceride Oils[1]

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Efficient continuous methanolysis using an alkaline catalyst is achieved in the simple system described.

At 100°C., in one hour, a volume of oil equal to six times the free volume of the reactor is converted to methyl esters.

Un reacteur simple à haut rendement horaire pour la méthanolyse continue en milieu alcalin est décrit.

A 100°C. le volume d'huile convertie par heure égale 6 fois le volume libre du réacteur.

A three-step process for the production from herring oil of methyl ester fractions having specific properties was described by the authors in 1957[1]. The process comprised: first, the production of mixed methyl esters by methanolysis of the oil; second, their fractionation by solvent segregation; and third, the fractional distillation of the segregated ester fractions. Since steps 2 and 3 lend themselves to continuous operation, it seemed desirable, both from the point of view of economy and of simplicity, to integrate the three steps in one continuous process. Continuous methanolysis was therefore investigated.

The reaction expressed by the following equation

$$\text{Triglyceride} + \text{methyl alcohol} \overset{OH^-}{\rightleftharpoons} \text{glycerol} + \text{methyl esters}$$

had been known for many years as a typical example of alcoholysis. As such it had been described and discussed in several articles which have been reviewed recently by Kaufmann *et al*[2], and by Poré[3].

In many laboratories, the reaction was applied to batch type operations only. The authors themselves had used extensively the conditions described by Bradshaw and Meuly[4] whereby over 95% of a glyceride oil can be converted to methyl esters in about one hour. The reaction was carried out at 60°C. using 75% excess methanol and 0.5% NaOH as the catalyst.

The above conditions had been found very satisfactory in promoting the separation from the final reaction mixture of two immiscible phases, the methyl ester layer and the glycerol layer, thereby simplifying the recovery of the products. Increasing the excess methanol beyond 100% was observed to interfere with this separation, and likewise, concentrations of the catalyst above 1% were found to complicate subsequent operations.

It was thought that a simple solution to the problem of continuous methanolysis might consist of pumping metered amounts of the reagents through a heated channel allowing sufficient time of contact for a satisfactory degree of methanolysis to develop in the mixture.

Evidently, the efficiency of such a system would be increased by an increase of the reaction rate. For the reasons given above, means of attaining this result were restricted insofar as excess methanol and amount of catalyst were concerned. The effects of an elevation of the temperature, on the other hand, were unknown.

The results of kinetic study of the reaction, which will be presented in detail elsewhere, indicate the following useful facts.

An appreciable increase in reaction rate can be achieved by operating at higher temperature. In Figure 1 is shown a curve expressing the relationship between the temperature and the time required to promote 90% release of the glycerol from herring oil using 0.5% NaOH and 70% excess methanol. It was found that the final equilibrium composition did not change within the range of temperature explored. Under the conditions just described, a maximum release of the glycerol corresponding to 95% was obtained in less than 7 minutes at 120°C.

The reaction was also found to involve the consecutive equilibria described by the following equations.

$$\text{Triglyceride} + \text{MeOH} \overset{OH^-}{\rightleftharpoons} \text{diglyceride} + \text{methyl ester}$$

$$\text{Diglyceride} + \text{MeOH} \overset{OH^-}{\rightleftharpoons} \text{monoglyceride} + \text{methyl ester}$$

$$\text{Monoglyceride} + \text{MeOH} \overset{OH^-}{\rightleftharpoons} \text{glycerol} + \text{methyl ester}$$

As these equations indicate, percentage conversion to methyl ester will be higher than percentage glycerol release.

It was also found that continuous agitation of the reaction mixture did not decrease the time required to reach final equilibrium. Indeed, no agitation is necessary after an initial thorough mixing of the reagents.

In designing the simple unit described below, we gave the reactor dimensions estimated to correspond to an output of 225 g. methyl esters per minute at 110°C., in order to match the capacity of the solvent segregation unit used in the following step of our process[1].

[1]Manuscript received November 4, 1960; accepted March 20, 1961.
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Contribution from the Fisheries Research Board, Technological Station, Halifax, N.S.

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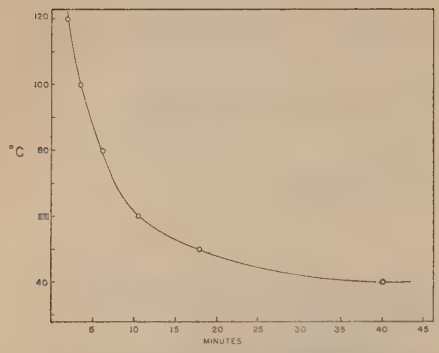

Figure 1—Curve expressing the relationship between the temperature and the time required to promote 90% release of the glycerol from herring oil using 0.5% NaOH and 70% excess methanol.

Allen and Kline have patented a procedure for the continuous methanolysis of glyceride oils[5], a general purpose continuous reactor has been patented[6], and a continuous laboratory unit for the conversion of acidulated soapstock using acid catalyst was described by Eaves *et al.*[7].

Description of the Unit

As shown in Figure 2, a solution of the catalyst in absolute methyl alcohol is contained in tank A. This solution, and the oil stored in tank B are pumped simultaneously through the system, respectively by pumps C and D. The latter are actuated by a common adjustable speed motor drive E. The liquids are mixed in F where they form an emulsion. F is a shallow circular box containing a magnetic stirrer actuated by a motor-driven external magnet. The bolted lid of this box is fitted with a thick glass window allowing visual inspection for proper adjustment of the stirring speed. The latter should be sufficient to emulsify the mixture and maintain the box full of emulsion. The reaction mixture reaches preheating coil G, a 50-ft. length of coiled ¼-in. internal diameter stainless steel tubing. This is followed by H, the reactor proper, a 50-ft. length of coiled ¾-in. internal diameter stainless steel tubing filled with small glass Raschig rings, the function of which is to promote efficient heat exchange through agitation. Both coils are immersed in oil bath I, which is equipped with electric immersion heaters, stirrer, temperature indicator and bimetallic thermoregulator. The latter, connected to a relay and switch combination, allows ±0.5°C. adjustment of the bath temperature. The temperature of the reaction mixture on entering the reactor is read on the recorder connected to thermocouple R. This temperature is about 3°C. below the bath temperature.

The reaction mixture then passes through cooling coil K, 50 ft. of ¼-in. internal diameter stainless steel tubing fitted in water jacket L, the flow being adjusted to bring the product to about 25°C. Spring valve M is adjusted to a pressure appreciably above the boiling point of the reaction mixture, i.e., 20 lb. above the vapor pressure of pure methanol at the temperature of the experiment. Through valve N, the reaction mixture reaches centrifuge O (Sharples motor-driven super centrifuge) where the methyl ester phase (outlet P) and the glycerol phase (outlet Q) are separated.

Operational Data

After adjusting the bath temperature and the operating pressure (valve M), three-stage metering pumps C and D are adjusted separately (stroke length) to deliver the required amounts of oil and catalyst solution at the selected motor speed.

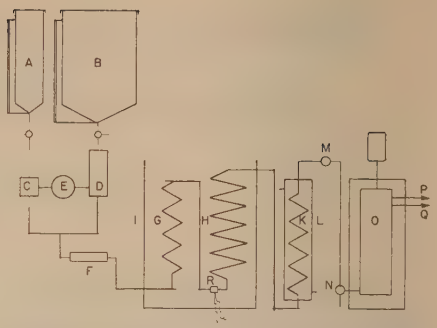

Figure 2—Pilot plant unit for the continuous methanolysis of glyceride oils.

The liquids can then be pumped in simultaneously.

A 100-ml. sample of product is collected at intervals from 3-way valve N in a container containing enough mineral acid to neutralize the catalyst. Free glycerol is then determined by a standard method on the chloroform extracted mixture[8]. Constancy of the glycerol value is observed after 30 min. Valve N can then be connected to O, and the glycerol and methyl ester phases collected separately.

Further processing of the methyl ester phase consists of vacuum stripping of the methanol it contains, and separating the small amount of soap which then precipitates. The glycerol phase is processed for the production of pure anhydrous glycerol according to a procedure which will be described in a separate article.

Our work with this unit has consisted so far in establishing its efficiency under conditions of normal production. The data thus far collected therefore do not involve a wide range of conditions.

Dried oils were used throughout these experiments since it was established that moisture will interfere very markedly with the reaction at elevated temperatures.

Some typical results shown in Table 1 have been obtained with seal oil having the following characteristics: F.F.A., 1.5%; I.V., 146; total glycerol, 10.02%. With this oil, the maximum value observed for glycerol release was 93% in a batch operation after 24 hr. at 60°C. (100% excess methanol, 0.5% NaOH).

A comparison of this result with those listed in Table 1 is legitimated by the simple rules governing equilibrium reactions. It is well known that the final equilibrium composition is unaffected by catalyst concentration. Further, the equilibrium will be displaced by a larger excess of methanol towards higher levels of conversion. Finally, it has been established, as stated

TABLE 1

GLYCEROL RELEASED FROM SEAL OIL IN RELATION TO TEMPERATURE, TIME OF CONTACT, EXCESS METHANOL, AND CATALYST CONCENTRATION

t °C.	Total flow ml./min.	Time of contact min.	Excess MeOH %	NaOH %	Free glycerol %
90	356	8.4	78	0.64	91.3
90	440	6.8	75	0.64	91.2
100	369	8.1	85	0.67	92.4
110	363	8.25	94	0.70	91.3
120	369	8.1	95	0.70	90.5

above, that the degree of conversion was unaffected by the temperature used within the range of temperature explored. For these reasons, the 93% conversion obtained after 24 hours at 60°C., undoubtedly an equilibrium value, and obtained with the largest excess methanol applied in this study, must correspond to the maximum degree of conversion attainable with this oil within the range of conditions used.

In Table 1, excess methanol is calculated as percentage over theoretical amount estimated from total bound glycerol. The catalyst used is expressed as percentage of oil weight. The time of contact is determined by dividing the free volume of the reactor (3000 ml.) by the total volume of reaction mixture flowing per minute.

It appears that a degree of conversion approaching the maximum attainable is achieved within a time of contact predictable from the kinetic data. The efficiency appears to fall to some extent when the temperature rises above 100°C. At this temperature the yield in methyl esters is about 260 gm./min. or somewhat in excess of the expected output. The volume of oil which can be treated in 24 hr. is 144 times the free volume of the reactor, an indication of the remarkable efficiency of this simple system.

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Recovery and Purification of Glycerol From Methanolysis

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In the past few years the importance of methyl esters for the production of fatty alcohols, monoglycerides . . . etc. has been increasing.

Methanolysis, described by the following equation

$$\text{Triglyceride} + \text{MeOH} \underset{\text{Catalyst}}{\overset{(OH^- \text{ or } H^+)}{\rightleftarrows}} \text{Methyl esters} + \text{glycerol}$$

is a particular case of the more general reaction known as ester-interchange which has been reviewed repeatedly over the last two decades and more recently by Kaufman *et al.* (1) and by Poré (2).

As early as 1944, Wright *et al.* (3) had already pointed out the potentiality of this reaction for the treatment of glyceride oils and had indicated advantages that might be derived from its application.

Hydrolytic processes leading to the formation of acids, the Twitchell process for example, are still used to a considerable extent and a large fraction of the total glycerol produced in the world is derived from their use.

From the point of view of glycerol manufacture, one of the advantages of the methanolysis process over others is that it is anhydrous and thereby avoids the largest part of the costs involved in glycerol recovery from hydrolysis, i.e., the cost of concentrating dilute aqueous solutions of glycerol. Such costs are the result not only of the high calorific requirements, but also of the costlier equipment, maintenance, and manpower involved in handling comparatively much larger volumes per pound of product.

The conversion of glyceride oils to methyl esters, on the other hand, enlarges rather than restricts the use of fatty components and does it in two ways. Firstly it permits a simpler and more efficient fractionation than is possible with fatty acids, thereby increasing the variety of byproducts that can be derived from a given oil. Secondly, the methyl esters themselves are often more easily converted than fatty acids to interesting products. The authors have exemplified these facts in a previous article(4) describing a process for the production of methyl esters from marine oils comprising the three following steps:

Step A. Methanolysis producing crude, mixed esters.

Step B. Liquid-liquid segregation of the mixed esters.

Step C. Distillation, leading to methyl ester fractions possessing contrasting characteristics.

Since steps B and C are continuous processes, a streamlining of the whole operation resulting in more economical manufacture depends on continuous methanolysis being the first step. This was achieved by the successful operation of a continuous reactor, described in a recent article, having an hourly output equivalent to six times its own volume(5).

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An important point regarding the compactness and simplicity of installations based on methanolysis is demonstrated in this study by the fact that a 2-gallon reactor could produce one million pounds of methyl esters yearly. Only alkaline catalysts, such as NaOH, were used in this study.

Further studies included the recovery of glycerol, one of the two major products of the reaction. Here again a process adaptable to low cost continuous operation resulting in the highest yield of high quality glycerol was sought.

The results of this research are described in the present paper.

The Glycerol Phase

The continuous production of methyl esters by the process previously described(6) involves the centrifugal separation of the reaction mixture in two phases. The methyl ester phase, a pale yellow liquid from which excess methanol is stripped, and from which some soaps are removed, is submitted to continuous segregation. The glycerol phase is a dark, viscous liquid from which glycerol is produced.

Typical data for the methanolysis of herring oil are given in Table 1 and 2.

The percentage composition of the two phases given in Table 2 shows that, save for the presence of glycerol, the glycerol phase has little in common with the "sweet liquors" and other similar solutions used as the starting point for glycerol recovery in hydrolytic processes; the

TABLE 1
METHANOLYSIS OF HERRING OIL IN REACTOR
Input to reactor

100 lb herring oil		19 lb methanol	
Iodine value	137	76% excess	
Free fatty acid (as oleic)	1.1%	99.5% reagent grade	
Saponification value	189 mg KOH/g		
Total glycerol	10.1%	0.65 lb NaOH dissolved	
Reactor temperature	100°C.	0.65% to oil	
Reactor pressure	100 psi		
Contact time	7 min		
Throughput	440 ml/min		

TABLE 2
COMPOSITION OF PRODUCTS FROM REACTOR
Output from reactor

Glycerol phase 20 lb		Methyl ester phase 99 lb	
Glycerol	9.2 lb	Methyl esters	93 lb
Methanol	4.5 lb	Soaps	0.5 lb
Methyl esters, soap, glycerides, colour etc.	6.3 lb	Methanol	4.0 lb
		Glycerides (mono,di,tri)	2.0 lb
		Glycerol	0.10 lb

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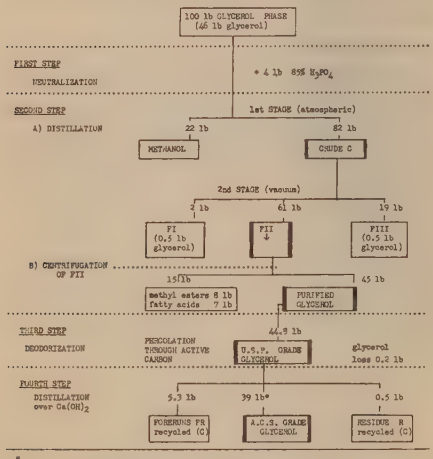
major difference being of course the absence of water. This precludes the use of methods devised especially for the purification of aqueous solutions of glycerol[6-8], as well as those described in a recent review[9]. Several methods have also been described for the purification of concentrated glycerol but aside from that of Price and Sprules[10] there are no published data concerning the treatment of the glycerol phase obtained by alkaline alcoholysis.

Since distillation is an important step in the method of purification to be described, the other most relevant data are those concerning glycerol distillation recently reviewed by Stage[11].

Outline of the Recovery Process (see Figure 1)

The first step consists of neutralizing the catalyst and freeing the fatty acids combined as soaps. To this end, 85% orthophosphoric acid is added in slight excess calculated from the amount required to bring a dilute sample to pH 6.



*Actual yield with recycling of FR and R: 44 lb

Figure 1—Outline of recovery process.

The second step consists of a two-stage distillation of the neutralized material, the first consisting of a stripping of methanol under atmospheric pressure. The second stage, carried out under vacuum, yields crude glycerol. The major impurities are methyl esters and fatty acids which are separated from the glycerol by centrifugation yielding a colorless, but not odorless, product.

The third step consists of percolating this glycerol over granulated activated carbon. The product is water-white, odorless glycerol answering the U.S. Pharmacopeia specifications[12].

A fourth step is required if C.P. glycerol is to be produced. This consists of a two-stage vacuum fractional distillation, carried out in the presence of a small amount of Ca or Ba hydroxide, further described under "experimental". The foreruns, comprising about 10% of the total input, are recycled with the methanol-free crude in the second step. The remaining 89% of the distillate meets the American Chemical Society (A.C.S.) specifications for C.P. glycerol. The residue is recycled with the foreruns.

Experimental

Analytical Procedures

The major impurities in glycerol obtained at the various stages of purification starting with the crude product from methanolysis are fatty acids and methyl esters, the molecular weights of which average those of oleic acid and methyl oleate, respectively.

The method used for their determination in the products just described is that of the U.S. Pharmacopeia[12], giving a value E expressing the millilitres of 0.5 N NaOH consumed in the saponification of a 50-g sample of test material. The specification for U.S.P. glycerol is that this value should not exceed 1 ml.

The specifications for A.C.S. Reagent Grade glycerol, as accepted by the Society (Annual Meeting, June, 1953)[13] refer to fatty acid ester impurities (expressed as glycerol butyrate) by setting the limit at 0.07%.

In the present work, W, the weight percent of impurities (acid + esters) was calculated from values obtained by using the U.S.P. method of determination, converting the millilitres 0.5 N NaOH (E) found to their methyl oleate equivalent for 100 g of test material (E × 0.296).

TABLE 3
COMPARISON OF GLYCEROL QUALITIES

	U.S.P. glycerol	C.P. A.C.S. Reagent	Crude glycerol after 1st distillation	Glycerol after carbon treatment	Glycerol after 2nd distillation over $Ca(OH)_2$
Colour	pass test	pass test	pass test	pass test	pass test
Odour	slight	slight	unpleasant	slight	slight
Specific gravity (25°/25°)	>1.249	>1.249	1.259	1.260	1.261
Residue on ignition	0.01%	0.005%	0.008%	0.003%	0.001%
Readily carbonizable substances	pass test	pass test	will not pass test	pass test	pass test
Fatty acids and esters E*	1		0.74	0.55	0.07
Fatty acids and esters W**		0.07	0.22	0.16	0.020
Fatty acids and esters S***			0.03	0.02	0.002
% glycerol by assay	>95%	>95%	98.5%	99.0%	99.5%
Neutrality	pass test	pass test	pass test	pass test	pass test
Silver reducing substances	"	"	"	"	"
Chloride	"	"	"	"	"
Sulfate	"	"	"	"	"

*E = ml 0.5 NaOH/50 g test material (U.S.P. specifications)
**W = weight % impurities, see text (A.C.S. Reagent Grade)
***S = saponification value (percent Na_2O), ("Dynamite" specifications)

Saponification values, S, were also obtained by the method prescribed for Grade B, High Gravity (Dynamite) glycerol. The latter are expressed as weight percent of Na_2O.

Table 3 gives these values for all products described below along with the results of other tests carried out as prescribed by official specifications.

Table 4 gives the composition of some of the fractions shown in Figure 1.

Laboratory distillations of the neutralized glycerol phase were carried out in batches of 400-500 ml in an apparatus fitted with a standard 40 cm Vigreux column at a rate of 100 ml/hr. Methanol was recovered at atmospheric pressure, a slight vacuum being applied near the end of the distillation.

A cold trap was included in the apparatus. The second stage of the distillation was carried out in the same apparatus under 5 mm Hg pressure.

TABLE 4
COMPOSITION OF FRACTIONS SHOWN IN DIAGRAM 1

	FI	FII	FIII
	lb	*lb*	*lb*
Glycerol	0.5	45.0	0.5
Methanol	0.6	–	–
Water	0.2	–	–
Lower acids, aldehydes etc.	0.7	–	–
Methyl esters	–	8.5	–
Fatty acids	–	7.5	–
Dark, viscous residue	–	–	14.0
Sodium phosphate	–	–	4.0
Totals	2.0	61.0	19.0

TABLE 5
TYPICAL RESULTS OF DISTILLATION IN FOURTH STEP SHOWING EFFECT OF REFLUX. (STARTING MATERIAL: 100 LB U.S.P. GLYCEROL CONTAINING 0.16% FATTY ACIDS AND METHYL ESTERS; 0.5 LB CALCIUM HYDROXIDE ADDED)

	Refluxed 30 min, distilled		Not refluxed, distilled	
	Yield	Fatty impurities	Yield	Fatty impurities
	lb	*%*	*lb*	*%*
Foreruns	11.8	0.5	10.7	0.9
A.C.S. Grade glycerol	87.0	0.02	88.3	0.05
Residue	2.5	3.2	1.3	1.5

Pilot plant distillations were carried out in an apparatus equipped with a 3-in. Oldershaw bubble cap column 29 in. long featuring 10 plates. This was mounted on a 3-gallon stainless steel pot. A reflux ratio of 1:2, (Glenlab Ratio Control) was used, and the rate of distillation was 2 lb/hr.

Vacuum (5 mm Hg) was maintained by a National Research Corporation (N.R.C.) Rotary Gas Ballast pump fitted with a cold trap.

After methanol stripping, the first fraction (FI) collected under vacuum consisted of some water, a small amount of residual methanol, methyl esters of lower molecular weight and aldehydes. The main fraction (FII) separated in two layers, the fatty acids and methyl esters floating on the glycerol. Effective separation of the two phases was obtained by centrifugation.

The third step was carried out by passing the glycerol obtained as described above over a column of granulated carbon. The efficiency of the system was studied by continuously comparing the content in impurities before and after treatment. From these experiments the amount of carbon necessary to bring about complete deodorization was estimated to be less than 0.1% of the glycerol treated. Glycerol losses resulting from the treatment (assuming spent carbon being discarded) were estimated at less than 0.5%. This operation also resulted in a reduction of combined methyl ester and fatty acid content from 0.22 to 0.16%.

In this operation, Pittsburgh Coke and Chemical Company, type Cal 12 × 40 mesh was found effective.

The treatment for the production of C.P. glycerol consisted of heating under reflux (160 - 170°C) the U.S.P. Grade glycerol obtained by carbon treatment for 20 to 30 minutes with 0.1 to 0.5% calcium or barium hydroxide. This was followed by fractional distillation under 5 mm Hg. The effect of this treatment is illustrated in Table 5 showing the combined concentration of fatty acids and esters in distillates obtained under the same conditions with and without reflux. The substantial reduction in forerun impurities is due partly to a conversion of methyl esters to monoglycerides and of fatty acids to soaps, both of which are less volatile and accumulate in the tails.

The latter, along with the foreruns (10%) are recycled, being returned to the crude, methanol-free glycerol. If the refluxing stage is omitted, the resulting product will not be as pure (0.05% impurities instead of 0.02%) but will still meet the A.C.S. specifications.

Discussion

Losses in the process just described are held to a minimum. As shown in Figure 1, they occur in FI, FIII, and at the deodorizing stage (Step 3) and represent a total net loss of 2.3%. Thus about 97% glycerol is recovered from the glycerol phase.

On the other hand, only 93% of the total glycerol initially involved in triglycerides is released during methanolysis, the remaining 7% being found combined as mono-, di-, and triglycerides in residues from the distillation of the methyl esters. The largest part of these, i.e. at least two-thirds, is, however, recyclable. Assuming an additional 1% for handling losses, 5 to 6% would seem a reasonable estimate for the glycerol losses in the methanolysis process as a whole and a yield of 94 to 95% is therefore to be expected. As shown in Table 3, the specifications for U.S.P. and C.P. glycerol are met by the products.

In this process, volumes and number of operations are also kept to a minimum. The addition of concentrated orthophosphoric acid instead of other mineral acids also avoids corrosion problems either by these acids or their salts. All these features are in contrast with the process described by Price and Sprules.

Recoveries other than glycerol include the following. Leaching of FIII with water will recover most of the acid sodium phosphate having commercial value as fertilizer. The resulting fatty residue and the methyl esters and fatty acids recovered from FII are also valuable.

A minimum recovery of 96% in recyclable methanol was established for the total excess alcohol used in the methanolysis process. The net loss in methanol per 100 lb finished product (methyl ester or glycerol) is 7 lb. These, with 10 lb of orthophosphoric acid and 0.1 lb of granulated carbon represent the reagents spent in producing 100 lb of glycerol.

The four steps described above are easily adaptable to a continuous operation forming an uninterrupted sequence

with the production of methyl esters by the process already described by the authors[4].

Acknowledgment

The authors wish to thank R. St. C. Hiltz for conducting many of the analyses.

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